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Original Article

FORMULATION AND STORAGE STABILITY OF BETA CAROTENE ENRICHED VITAMIN D3 AND OMEGA 3 FORTIFIED COLD PRESSED VIRGIN COCONUT OIL

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ABSTRACT

Objective: The general objective of this study is to formulate beta carotene enriched, vitaminD3 and omega 3 fortified Cold Pressed Virgin Coconut Oil (CPVCO) and to analyze proximate composition and shelf life of beta carotene, vitaminD3 and omega 3 enriched CPVCO.

Methods: The CPVCO was prepared out of fresh full fat coconut flakes by cold pressed method. Vitamin D3, beta carotene, Cold Pressed Flax Seed Oil (CPFSO) and orange oil were added to CPVCO and carefully homogenized for 15 to 30 minutes. Formulated oil was packed in High Density Poly Ethylene (HDPE) bottles and stored at room temperature. The shelf life study of this oil was carried out for a period of 10 months.

Results: The proximate compositions of formulated CPVCO with Vitamin D3, β- carotene and CPFSO with regard to the proportion of fatty acids caprylic acid, myristic acid, steric acid, palmitic acid were all within the recommended standard limits in accordance APCC standard for formulated CPVCO. Whereas, lauric acid and capric acid were found to be slightly differ from APCC standard. However, the comparison of oil was concerned, the original CPVCO was amounting to 88.5% and the remaining were 10% of CPFSO and 1.5% orange oil. 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.

Conclusion: In the present study, it was demonstrated that CPVCO was a suitable medium for producing value added functional oil. Apart from being healthy oil, CPVCO may also be used as a nutraceutical product.

Keywords: Coconut flakes, β- carotene, Vitamin D3, CPFSO, CPVCO.

INTRODUCTION

Virgin Coconut Oil (VCO) is one of the Value Added Products (VAP) to coconut, which has numerous proven applications in medicine, food, cosmetics etc. [1, 2]. In some animal studies, VCO has been shown to reduce total Cholesterol, Triglycerides, Phospholipids, Low Density Lipoproteins (LDL), Very Low Density Lipoprotein (VLDL) levels and increasing the High Density Lipoproteins (HDL) in serum and tissue were reported [3]. VCO showed significant anti thrombotic effect in animal studies, where animals fed with VCO, antioxidant vitamin levels were also increased [4].

VCO incorporated in different essential oils (lemon, eucalyptus, lavender) were reported in the application of aroma therapy [5]. VCO has been demonstrated as high quality raw material for health and skin care products [6]. Researchers have also opined that VCO contains more phenolic compounds and antioxidant capacity than Commercial Coconut Oil [CNO] [7]. Some of the phenolic acids (protocatechic, vallinic, caffeic, syringic, ferulic and p-coumaric) were identified in VCO which were attributed for its antioxidant activity [8].

Presently, fortification and enrichment of the food items have been preferred in many of the developing countries to com bat many nutritional deficiencies. In many parts of the less industrialized world, vitamin A deficiency is a problem that affects nutritional status and health. In several countries, pro vitamin A carotenoids are the primary, if not the only, source of vitamin A [9– 12]. In humans, carotenoids and especially β-carotene of vegetables are an important source of vitamin A. Of 600 carotenoids from natural sources that have been characterized, fewer than 10% serve as precursors of vitamin A. β -carotene, the most nutritionally active carotenoid, forms 15% to 30% of the total serum carotenoids [13]. On the other hand, prevention of vitamin A deficiency through food fortification is a well recognized approach to solve nutritional problems, mainly in developing nations. Vegetable oil appears to be a suitable carrier for fortification or enrichment with vitamin A or beta-carotene as it is a daily fat source, high in energy, polyunsaturated fatty acids, and naturally occurring antioxidant vitamin E. Furthermore, processing of vegetable oil and technology of fortification with vitamin A or β -carotene are simple. Vitamin A and β -carotene are soluble in oils. Previous studies in the research done by (Favoro et al (1990), early in 1990's laboratory have shown refined soybean oil to be a good vehicle for vitamin A fortification [14].

Once foods were fortified with vitamin D the rickets which was appeared to have been conquered, many health care professionals thought the major health problems resulting from vitamin D deficiency had been resolved. Humans obtain vitamin D from exposure to sunlight, from their diet, and from dietary supplements [15-18].Solar ultravio-let B radiation (wavelength, 290 to 315 nm) penetrates the skin and converts 7-dehydrocholesterol to pre vitamin D_3, which is rapidly converted to vitamin D_3 because any excess pre vitamin D_3 or vitamin D_3 is destroyed by sunlight, excessive exposure to sunlight does not cause vitamin D_3 intoxication [15].

For over 80 years the importance of Poly Unsaturated Fatty Acid (PUFA) consumption for human health has been established. The FDA recently approved the use of Omega 3 PUFAs in supplements. Additionally, the market for Omega 3 PUFA ingredients grew by 24.3% last year, which affirms their popularity and public awareness of their benefits. PUFAs are essential for normal human growth; however, only minor quantities of the beneficial Omega 3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are synthesized by human metabolism [19]. As a matter of fact, the sources of omega 3 poly unsaturated fatty acids in India are rare and people have less access to this important nutrient intake. The primary sources of fishes are not available much in India except sardines in particular. Similarly the vegetarian sources like flaxseeds usage in the country are also less appreciable. Hence, in order to incorporate the antioxidant β-carotene and cardio friendly omega 3 PUFA in to easily and daily consumable vegetable oil was considered as the carrier or source. Thus in the present study an immense effort was taken to formulate the value added, enriched and fortified CPVCO.

The general objective of this study is to formulate beta carotene enriched and vitaminD3 and omega 3 fortified Cold Pressed Virgin Coconut Oil (CPVCO) and to analyze proximate composition and shelf life of beta carotene, vitaminD3 and omega 3 enriched CPVCO.

MATERIALS AND METHODS

Formulation of Beta Carotene Enriched and VitaminD3 and Omega 3 Fortified CPVCO

The 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 and the Cold Pressed Virgin Coconut Oil (CPVCO) was obtained.

Table 1: The Composition of Vitamin D3, Beta carotene, Omega 3 Fatty Acid and Orange Oil Enriched CPVCO

S. No.	Ingredients	Quantity (per 100 g)
1.	CPVCO	88.5 %
2.	CPFSO	10 %
3.	Orange oil	1.5 %
4.	Beta carotene	2.35 mg
5.	Vitamin D3	6000 IU

Vitamin D3 was purchased from Fermenta Biotech Limited, Thane, INDIA and that of beta carotene from the *dunaliella salina* algae from (Parry Neutraceutical, panangudi, TamilNadu, INDIA). Whereas, Omega 3 fatty acid (Prano Flax India Pvt Ltd (Cold Pressed Flax Seed Oil (CPFSO) and Orange oil were obtained from local market. Incorporation of the solvent extracted orange peel oil was considered in order to mask the dominant CPFSO flavor and secondarily to yield a pleasant colour and aroma to the resultant oil. Vitamin D3, beta carotene, CPFSO and orange oil were added to CPVCO and carefully homogenized for 15 to 30 minutes. The composition of vitamin D3, beta carotene, CPFSO and orange oil incorporated CPVCO are given in the Table No.1. Formulated oil was packed in High Density Poly Ethylene (HDPE) bottles and stored at room temperature. The shelf life study of this oil was carried out for a period of 10 months.

Proximate Composition of Beta Carotene Enriched and Vitamin D3 and Omega 3 Fatty Acid Fortified 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 [21].Arsenic, Cadmium, Mercury and lead were determined by using SO-CHML-CTS-C-01-QU-063 by ICPMS. The AOAC method (958.05, Association of official Analysis chemists, AOAC, 1990) with a few modifications was used to evaluate carotenoid content of oils.

Physico Chemical Properties

Total ash, peroxide value and acid insoluble ash percentage by weight were analyzed by the method suggested by AOAC (2005) [22]. 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 [23] [24] [25] [26]. 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)]** [22]. Whereas total plate count, coli form count, detection of Salmonella and yeast mold plate count were determined by ISO methods.

RESULTS AND DISCUSSION

Table 2: Proximate Composition of Natural Mixed Carotenoids from *Dunaliella salina* in edible oil

S. No.	Parameters	Specification	Results	Test Method
1.	Appearance	Oil	Oil	In house
2.	Colour	Reddish brown	Reddish brown	In house
3.	Loss on drying (%)	NMT* 1.0	0.48	USP<731> Loss on drying
4.	Total Carotenoids (%)	NLT* 2.5	2.6	Jenna gross, pigments in vegetables, chlorophylls carotenoids (100-101) by van nostrand reinhold, New York.
5.	Standard plate count (cfu/1g)	<1000	200	Bacteriological Analytical manual 8th ed, AOAC, USFDA, 1995.
6.	Yeast and mould (cfu/1g)	<100	20	Bacteriological Analytical manual 8th ed, AOAC, USFDA, 1995.
7.	Coliforms (/25)	Negative	Negative	Bacteriological Analytical manual 8th ed, AOAC, USFDA, 1995.
8.	E.Coli (/25)	Negative	Negative	Bacteriological Analytical manual 8th ed, AOAC, USFDA, 1995.
9.	Salmonella (/25)	Negative	Negative	Bacteriological Analytical manual 8th ed, AOAC, USFDA, 1995.
10.	Staphylococcus (/25)	Negative	Negative	Bacteriological Analytical manual 8th ed, AOAC, USFDA, 1995.

*NMT: Not More Than *NLT: Not Less Than

The carotenoids (from Dunallela Salina) in edible oil were assessed for various physico chemical and microbial parameters. On testing with standard protocol as given in the table, conversely revealed that the carotenoids satisfied the expected standards. Of course, even the bacteriological and fungi analysis showed no presence of any pathogens.

Table 3: Proximate Composition of Vitamin D3

S. No.	Test	Specification	Results
1.	Description	A clear yellow liquid	A clear yellow liquid
2.	Solubility	Practically insoluble in water, slightly soluble in anhydrous ethanol, miscible with solvents of fats	Complies
3.	Acid Value	NMT* 2.0	0.22
4.	Peroxide value	NMT* 20	1.17
5.	Vitamin D3 Assay	Between 3.6 to 4.4 MIU/g	4.20 MIU/g

*NMT: Not More Than *NLT: Not Less Than

From Table no.3, it is clearly understood that the physico- chemical assessment and vitamin D3 assay results certainly shows perfect compliance.

Table 4: Proximate Composition of Formulated Beta Carotene Enriched Vitamin D3 and CPFSO fortified CPVCO (g/100g) (mean ± SD)

S. No.	Test Parameters	Results	Test Method	APCC** standard For VCO
1.	Total fat %	99.79 ± 0.01	AOAC 18th Edition	-
2.	Energy	990 Kcal	Nutritive value of Indian foods, ICMR (Gopalan et al., 1996).	-
3.	Caprylic acid	7.2 ± 0.01	AOAC 18th Edition	4-10
4.	Capric acid	9 ± 0.2	AOAC 18th Edition	4-8
5.	Lauric acid	44 ± 0.03	AOAC 18th Edition	45-56
6.	Myristic acid	15.3 ± 0.3	AOAC 18th Edition	16-21
7.	Steric acid	1.8 ± 0.02	AOAC 18th Edition	2-4
8.	Palmitic acid	7.02 ± 0.01	AOAC 18th Edition	7.5-10.2
9.	Mono unsaturated fatty acid	5.57 ± 0.4	AOAC 18th Edition	-
10.	Poly un saturated fatty acid	2.8 ± 0.04	AOAC 18th Edition	-
11.	Beta carotene	2.35 ± 0.02	AOAC 18th Edition	-
12.	Vitamin D3	6000IU	AOAC 18th Edition	-
13.	Omega 3 fatty acid	5.67 ± 0.02	AOAC 18th Edition	-
14.	Omega 6 fatty acid	1.64 ± 0.01	AOAC 18th Edition	-
15.	Free fatty acid (as lauric acid) g/100 g	0.07 ± 0.03	AOCS Ca5a-40	Max 0.2
16.	Arsenic (as AS)	BLQ*(LOQ*: 0.05)	SO-CHML-CTS-C-01-QU-063by ICPMS	Max 0.1
17.	Cadmium (as Cd)	BLQ*(LOQ*: 0.01)	SO-CHML-CTS-C-01-QU-063by ICPMS	
18.	Mercury (as Hg)	BLQ*(LOQ*: 0.01)	SO-CHML-CTS-C-01-QU-063by ICPMS	
19.	Lead (as Pb)	BLQ*(LOQ*: 0.01)	SO-CHML-CTS-C-01-QU-063by ICPMS	Max 0.1

*BLQ: Below Limit of Quantification * Low Limit of Quantification, ** APCC: The Asian and Pacific Coconut Community

Fatty acid Composition of Formulated Beta Carotene Enriched Vitamin D3 and CPFSO Fortified CPVCO

The above Table no.4 depicts the proximate composition of formulated CPVCO with Vitamin D3, β- carotene and CPFSO with regard to the proportion of fatty acids caprylic acid, myristic acid, steric acid, palmitic acid where all within the standard recommended limits in accordance APCC standard for formulated CPVCO. Whereas, lauric acid and capric acid were found to be slightly differ from APCC standard. In fact the lauric acid content of the formulated CPVCO was 44 ±0.3 g per 100 g which when compared with our previous study (49 ± 0.06g/100 g) [Manikandan et al 2014] [27] is slightly lower. The reduction in the lauric acid content of this enriched and fortified oil may be attributed to the proportion of CPVCO. In the previous study lauric acid was estimated out of entire 100 gms of CPVCO only. Whereas, in the present study the original CPVCO was amounting to 88.5% and the remaining were 10% of CPFSO and 1.5% orange oil.

Though lauric acid content was less in the presently formulated oil yet comparable with the commercial coconut oils. Similarly the omega 3 PUFA content in the presently formulated oil was found to be highly appreciable (5.7 ± 0.02 g / 100 gms and omega 6 fatty acid was 1.64 ± 0.01). Whereas, the presence omega 3 PUFA in the plain CPVCO was negligible. With respect to heavy metals content namely Arsenic, Cadmium, Mercury and Lead were found to be below the level of quantified. On the whole the formulated oil was found to be highly safe and health oriented. In a study conducted by [Kamariah et al (2008)] [28], 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%.

Table 5: Physico Chemical Properties of Formulated CPVCO (g/100g) (mean ± SD)

S. No.	Testing Parameters	Cpvco	Test Methods	APCC standard
1	Total ash %	0.02 ± 0.1	AOAC 18th Edition, 2005	
2	Moisture %	0.28 ± 0.01	AOAC 18th Edition, 2005	
3	Iodine value	7.73 ± 0.03	AOCS Cd 1-25	4.1 – 11.00
4	Refractive index at 40 ^{0} C	1.48 ± 0.05	AOCS Cc 7-25	1.4480 – 1.4492
5	Saponification value	260 ± 0.02	AOCS 3-25	250 – 260
6	Specific gravity	0.92 ± 0.01	IS 548(part I): 1964	0.915 – 0.920
7	Peroxide value	Nil	AOAC 18th Edition, 2005	≤ 3 meq/ kg oil

Table 6: Assessment of Microbial Quality (cfu/g) for Fresh CPVCO

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

*Below the Limit Quantitation

Iodine Value (IV) is a measure of the degree of unsaturation in oil. The IV of CPVCO in fresh sample was 7.73 ± 0.03(Table.2). The values are similar to that was reported in literature [Marina et al 2009] [29] and [Henna et al 2009] [30]. 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 1.48±0.05. Whereas, [Kamariah et al 2008] [27], documented that of VCO was 1.44± 0.01. 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 263.8± 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] [31].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.

Table 7: Changes in Peroxide Value (mεq O_2 / kg fat) of Formulated 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	0.09	0.1	0.15	0.18	0.19	0.19	0.22	0.22
Specification	≤3	≤3	≤3	≤3	≤3	≤3	≤3	≤3	≤3	≤3

From the Table no 4, it was clearly evident that peroxide value of formulated 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)] stated that the standards for essential composition and quality factors of commercial VCO and its differentiation from Refined Bleach Deodorant (RBD) Coconut Oil and Copra Oil, standards for peroxide value should be <3.

Table no 8 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 8: Changes in Microbial Load (cfu/g) of Formulated 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

CONCLUSION

Though VCO has been as healthy oil worldwide, yet CPVCO has been recognized as a better medium for producing value added oil. CPVCO may also be used an excellent nutraceutical agent for specifically catering the required fat soluble nutrients to the needy. In the present, CPVCO enriched β- carotene and fortified with vitamin D3 and CPFSO proved to be an excellent nutraceutical agent.

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CONFLICT OF INTERESTS

Declared None

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