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Omega-6 and Omega-3 Polyunsaturated Fatty Acids of *Canarium zeylanicum* and Fourteen Additional Seeds from Sri Lanka

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Abstract

Canarium zeylanicum is an endemic plant, its fruit claimed to be consumed by animals such as wild boars and wild fowls. Seed oil of *Canarium zeylanicum* had been used in the past as edible oil, but currently it is used only in the Ayurvedic medicine. Seeds of *Canarium zeylanicum* are an excellent source of essential fatty acids, contains 53.6% linoleic acid and 23.21% linolenic acid with 52.22% of total fat. *Sesamum indicum*, *Anacardium occidentale* and *Arachis hypogaea* also showed higher oil contents of 55.71%, 49.05% and 48.34% respectively. According to the current nutritional recommendations, the ratio of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA) in human diet should be above 0.45, and the ratio of omega-6 to omega-3 fatty acids (ω6/ω3) should not exceed 4.0. All the examined seeds had the PUFA/SFA ratios in the recommended levels. The ω6/ω3 ratio of C*anarium zeylanicum* and *Limonia acidissima* were found to be 2.3 and 0.6 respectively. Our results showed that the kernel of *Canarium zeylanicum* is a good and inexpensive source of omega-3 fatty acids and this seeds should be further investigated as a potential human/animal food source.

Keywords: *PUFA/SFA; omega-6/omega-3; Canarium zeylanicum; Limonia acidissima*

1. Introduction

All the animals including humans are capable of biosynthesizing monounsaturated fatty acids. However, animals do not have the necessary enzymes desaturases, which are essential to convert oleic acid into polyunsaturated fatty acids (PUFA), essentially linoleic and linolenic acids (Wallis *et al.,* 2002). Therefore, these essential fatty acids which are impossible to synthesize in the body, must be obtained from the diet. Fatty acid composition is one of the important factors that contribute to the development of cardiovascular disease (CVD) (Breslow, 2006; Lecerf, 2009), cancer (Harle *et al*., 2005; Fabian *at el.*, 2015) and degenerative diseases (Lorente-Cebrian *et al.,* 2015). It is considered that the increased consumption of monounsaturated fatty acids (MUFA) and PUFA, and decreased consumption of saturated fatty acids (SFA) are linked to positive health outcomes. The high consumption of SFA is associated with a high level of serum cholesterol and therefore related with CVD (Dietary guidelines advisory committee report, 2005). Many studies have highlighted that a balanced intake of PUFA/SFA is of paramount importance in regulating serum cholesterol levels. The recommended ratio of PUFA/SFA in human diet should be above 0.45 (Kang *et al*., 2005; Jakobsen *et al*., 2009). Most of

the edible oils such as sunflower, sesame, rapeseed, contain appropriate levels of PUFA and SFA as the ratio of PUFA/SFA are 5.0, 2.7, and 3.7 respectively (Vingering *et al.*, 2010). Coconut oil is rich in SFA (92.1%) with PUFA content of 1.6% (Orsavova *et al.*, 2015). Therefore the ratio of PUFA/ SFA is 0.017 and other PUFA rich sources have to be consumed along with coconut oil. The main plant essential fatty acids ω3 and ω6 are commonly in the form of α-lnolenic acid (C18:3$^{\Delta 9,12,15}$, ALA) and linoleic acid (C18:2$^{\Delta 9,12,}$, LA). Excessive amounts of ω6 and a very high ratio of ω6/ω3 is found to promote CVD, cancer, and autoimmune diseases, whereas increased levels of ω3 exert suppressive effects (Simopoulos, 2002; Simopoulos, 2008; Vingerine *et al.*, 2010). Several sources suggest that ratio of ω6/ ω3 fatty acids should not exceed 4.0 (Nile & Park, 2013; Simopoulos, 2016). Oils of canola, rapeseed, and cannabis are reported to contain desirable ratios of ω6/ ω3 as 1.8, 2.4 and 3.7 respectively. The same ratio of other oils such as sunflower, olive, safflower and sesame, are reported to be 8.5, 10, 78.6 and 99.9 respectively (Carvalho *et al.*, 2006; Vingerine *et al.*, 2010).

In our study, fifteen varieties of seeds with different importance have been studied for their total lipid content and the ratios of PUFA/SFA and ω6/ω3. The seeds examined in the present study were selected with the aim of finding healthy and economical fatty acid sources for human and animals.

2. Materials & Methods

2.1 Sample collection and identification

The scientific names, vernacular (Sinhala) names and English name of selected plants are listed in Table 1. Mature seeds were collected from known localities in the dry and wet zones of Sri Lanka. Some seeds were purchased from Ayurvadic pharmacies in Kandy. Seeds were identified by using herbarium specimens at the Royal Botanic Gardens, Peradeniya, Sri Lanka.

2.2 Analysis of total lipid content of seeds

Lipid extraction was carried out as described by Klara *et al*., 2002. Seeds were cleaned, washed with running water, and dried in an oven at 50^{o}C to a constant weight. Seeds were ground into a fine powder using a SISIL mixer grinder. Triplicates of 100g of seed powder were sonicated in 200ml of hexane using an ultrasound sonicator (VWR Ultrasonic Cleaner USD 1700) at 50^{o}C for 2 hours. The extraction procedure was repeated thrice and the collected solvent was removed using a rotary evaporator (LABOROTA 4000, Heidolph) at 40^{o}C. The oil extract was further dried in a vacuum oven (Vacutherm, HERAEUS INSTRUMENTS) to a constant weight, and the weights of the extracts were measured.

2.3 Analysis of fatty acid composition

2.3.1 Preparation of FAME by direct trans-esterification

Methyl esters of fatty acids were prepared by direct trans-esterification as described by Yaniv *et al*, 1991 with slight modifications. Duplicate samples of seed powder (200mg) were mixed with 0.3ml dichloromethane and 2.0ml of 0.5M sodium methoxide in a screw capped tube. Tubes were heated at 50^{o}C for 30 min. in a heat block and the reaction was stopped by adding 5ml of distilled water containing 0.1ml of glacial acetic acid. The esterified fatty acids (FAME) were extracted into 0.5 ml of hexane, and the hexane layer was separated by centrifugation (1500 rpm for 10 min) at 5^{o}C. FAMEs in hexane were stored at -20^{o}C until they were analyzed by GC.

FAME of authenticated samples of palmitic acid (16:0), Stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) were prepared by the same procedure using 5mg each.

2.3.2 Gas Chromatography of methylated fatty acids.

A fused silica gas chromatography capillary column (SUPELCO-2560, 0.2 µm film thickness, 100m x 0.25mm) was used in a gas chromatograph (Shimadzu gas chromatograph GC-9AM) equipped with a flame ionization detector. The flow rate of the carrier gas Helium was 30ml/ min and the column temperature was 200^{o}C. Detection and injection temperatures were maintained at 250^{o}C throughout the analysis. Authenticated samples were analyzed under the same GC conditions and fatty acids in seeds were identified by comparing with the retention times of the standards and quantified by the relative peak areas. The fatty acid composition of each sample represents the mean of three replicate gas chromatographic analyses.

3. Results & Discussion

Total fatty acid content of tested seeds is given in Table 2. The total fat percentage of seeds ranged from 0.93% to 55.71%. *Sesamum indicum* showed the highest oil content (55.71%) while *Canarium zeylanicum* (*CZ)* (52.22%) *Anacardium occidentale* (49.05%) and *Arachis hypogaea* (48.34%) were among the other seeds with high oil content. The results of the fatty acid composition of analyzed seeds are summarized in Table 2. The distribution of fatty acids of *Sesamum indicum* was 43.67% oleic acid, 39.2% linoleic acid, 7.92% palmitic acid and 4.64% stearic acid. Uzun *et al.,* 2008 reported that the oil content of *Sesamum indicum* varies between 41.3 to 62.7%, and the percentages of linoleic, oleic, palmitic and stearic acids in the seed oil ranged between 40.7-49.3, 29.3-41.4, 8.0-10.3 and 2.1-4.8 respectively. *CZ* an endemic plant to Sri Lanka, is an excellent source of essential fatty acids, containing 53.61% linoleic acid and 23.21% of linolenic acid. Seneviratne and Kotuwagedara (2009) reported 49.35% linoleic acid and 19.0% linolenic acid in *CZ*. Fat is the major macronutrient present in *Anacardium occidentale* accounting for 49.05% of the total dry weight. Oleic acid was the most abundant with a contribution of 62.17 % to the total fat content, followed by linoleic acid (21.54%), palmitic acid (10.98%), stearic acid (5.93%) and linolenic acid (0.53%). Similar fatty acid compositions were reported by Rico *et al.,* 2016 in cashew kernels from different origins. The major fatty acids present in *Arachis hypogaea* L. are oleic, linoleic and palmitic as shown in Table 2. These results are in accordance with literature (Nile and Park, 2013). Oil content of the seeds of *Manihot glaziovii* was 18.6%, calculated without the seed coat. Alves *et al.,* 2014 reported that the total oil content in *Manihot glaziovii* falls in the range of 14.1 – 25.1%. The most abundant fatty acid was linoleic acid (64.71%) followed by palmitic (13.91%), oleic (6.75%), stearic (4.63%) and linolenic (1.27%). According to Alves *et al.,* 2014, the composition of fatty acids in descending order ,was linoleic acid (62.5%) oleic (19.3%), palmitic (13.5%) , stearic (3.5%) and linolenic (1.3%). Unlike most beans, soybeans contain a very high proportion of fatty acids, in the range 16.8-18.8% (Milinsk *et al.,* 2007) in different varieties of soybeans. In this study total fatty acid content of *Glycine max* was 17.7%, with 53.67% linoleic acid, 22.16% oleic acid, 12.42% palmitic acid, 4.91% linoleic acid and 3.17% stearic acid. Fatty acids in *Glycine max* were reported as linoleic (55-59%), oleic (16-19%), palmitic (12-14%), linolenic (6-9%) and stearic (3-4%) acids (Milinsk *et al*., 2007). Total oil content of seeds of *Limonia acidissima* was not previously reported, but fruit pulp contained 4.38% fat (Panday *et al*., 2014). According to the present study, this seed is a good dietary source with 12.5% total fat. It is a rich source of unsaturated fatty acids, containing 32.33% linolenic acid, 29.06% oleic acid, and 19.75% linoleic acid. Seeds of *Leucaena leucocephala* were low in total oil with a 6.28% yield. The oil is rich in linoleic acid (63.52%), and other fatty acids present are 16.5 % palmitic acid and 14.6% oleic acid. Linoleic acid levels were reported in the range 50-54% (Imededdine, *et al.,* 2014). Millets are reported as sources of linoleic, oleic and palmitic acids (Cepkova et al., 2014). In our study, *Setaria italica* gave the highest amount of linoleic acid content of 75.88%, followed by *Panicum miliaceum* L. of 67.8% with total fatty acid levels of 4.96% and 2.93% respectively. The content of oleic and palmitic acids reached 17.18% and 3.58% in foxtail millet and 25.16% and 3.24% in proso millet respectively. Cepkova *et al.,* 2014 reported the content of linoleic, oleic and palmitic acids in the range of 68-71%, 15-18% and 7-9 % respectively. *Dolichos biflorus* and *Vigna unguiculata* (L.) Walp. are protein rich sources but their total lipid content is comparatively very low with 1.1% and 0.93% respectively.

According to the current nutritional recommendations, the ratio of PUFA/SFA in human diet should be above 0.45 and fatty acids ratio of ω6/ω3should not exceed 4.0 (Kang 2005; Nile & Park 2013). In our study, all the examined seeds are found to contain the recommended levels of PUFA/SFA in the range 1-21.4 as shown in Table 2. The ratios of ω6/ω3 in *CZ, Vigna unguiculata* and *Limonia acidissima* were within the recommended level as 2.3, 1.9 and 0.6 respectively. *CZ* is a potential source of fat, containing 52.22% total fat, high omega3 fatty acid content and low ω6/ω3ratio, and would be an excellent source in providing high amounts of ω3 edible oil. Although this oil was consumed in the past, it is not been used as an edible oil at present. The plant *CZ* is classified as vulnerable in the IUCN red list of threatened species (2011). Therefore precautions have to be taken to protect this plant. Measurable amount of linolenic acid was not detected in the seeds of *Sesamum indicum*, *Panicum miliaceum* and *Leucaena leucocephala. Sesamum indicum* is one of the oldest edible oil crops and it is widely used in different food preparations. It cannot

be used as a single source of fatty acids in human nutrition, and has to be combined with other oils rich in omega-3 fatty acids. The major fatty acid of seeds of *Limonia acidissima* was omega-3 linolenic acid and therefore seeds have to be included in all food preparations to improve the nutritional value.

4. Conclusion

Results of the present study indicate oil of *CZ* and *Limonia acidissima* are rich sources of omega-3 fatty acids. Since the total fatty acid content of the kernel of *CZ* is more than 50%, it may be used to make different oil combinations by mixing with other oils which are poor in essential fatty acids. Further seeds of *Limonia acidissima* are recommended to incorporate in all food preparations of wood apple to enrich the nutritional value.

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Table.1 Scientific name, local name and the English name of seeds.

Plant species	**Common name in Sinhala**	**Common name in English**	**Family**
Anacardium occidentale L.	Kaju	Cashew nut	Anacardiaceae
Arachis hypogaea L.	Rata-kaju	Ground nut	Fabaceae
Canarium zeylanicum (Retz.) Bl.	Kekuna	-	Burseraceae
Dolichos biflorus L.	Kollu	Horse gram	Leguminoseae
Glycine Max (L.) Merr.	Soya	Soybean	Leguminoseae
Leucana leucocephala (Lam.) de Wit.	Ipil ipil	White lead tree	Leguminoseae
Limonia acidissima L.	Divul	Wood apple	Cucurbitaceae
Manihot glaziovii (Mull.) Arg.	Gas manyokka	Indian rubber	Compositae
Mucuna pruriens (L.) DC.	Achariapalu	Cowhage	Leguminosae
Panicum milliaceum L.	Meneri/ Hin-meneri	Little /Proso millet	Poaceae
Psophocarpus tetragonolobus (L.) DC.	Dambala	Winged bean	Lauraceae
Sesamum indicum L.	Thala	Sesame/Gingerly	Myrtaceae
Sesbania grandiflora (L.) Poiret.	Kathurumurunga	Hummingbird Tree	Fabaceae
Setaria italica (L.) Beauv.	Thanahal	Foxtail millet	Poaceae
Vigna unguiculata (L.) Walp.	Cowpea	Cowpea	Fabaceae

Table. 2 Composition (%) of different fatty acids and their ratios

Plant	Total Fat %	Saturated Fatty acids		Unsaturated fatty acids			PUFA/SFA	ω6/ω3
		Palmitic %	Stearic %	Oleic %	Linoleic %	Linolenic %		
Anacardium occidentale L.	49.05 ± 3.96	10.98±0.72	5.93±0.22	62.17±1.39	21.54±2.25	0.53±0.21	1.3	40.6
Arachis hypogaea L.	48.34± 1.63	15.55±0.45	ND	46.96±0.84	33.78±0.20	0.314±0.18	2.2	108.9
Canarium zeylanicum (Retz.) Bl.	52.22 ± 2.08	5.84±0.92	1.23±0.44	16.08±0.07	53.61±0.29	23.21±0.55	10.9	2.3
Dolichos biflorus L.	1.1 ± 0.14	29.51±0.98	ND	17.04±0.30	43.92±0.09	9.03±0.47	1.5	4.86
Glycine max (L.) *Merr.*	17.78 ± 0.87	12.42±0.75	3.17±1.31	22.16±1.64	53.67±1.57	4.91±0.72	3.8	10.9
Leucaena leucocephala (Lam.) de Wit.	6.28 ± 0.69	16.52±0.27	ND	14.60±0.80	63.52±1.44	ND	3.8	-
Limonia acidissima L.	12.5± 1.12	13.92±0.45	ND	29.06±0.17	19.75±0.60	32.33±1.90	3.7	0.6
Manihot glaziovii (Mull.) Arg.	18.6 ±1.52	13.91±0.19	4.63±0.44	6.75±0.47	64.71±0.33	1.27±0.30	3.6	50.9
Mucuna pruriens (L.) DC.	4.2 ± 0.46	42.32±1.12	4.52±0.69	10.82±0.38	44.45±1.10	1.12±0.15	1.0	-
Panicum milliaceum L.	2.93 ± 0.17	3.24±0.37	ND	25.16±1.05	67.8±0.25	ND	20.9	-
Psophocarpus tetragonolobus (L.) DC.	16.3 ± 0.87	11.11±0.54	5.00±0.43	44.08±0.98	29.6±1.26	2.51±0.50	2.0	11.7
Sesamum indicum L.	55.71 ± 0.80	7.92±3.36	4.64±1.94	43.67±5.79	39.2±2.15	ND	3.1	-
Sesbania grandiflora (L.) Poiret	7.12 ± 1.36	15.58±1.72	3.02±1.05	11.66±1.87	64.26±4.85	7.84±1.33	3.9	8.2
Setaria italica (L.) Beauv.	4.96 ± 0.15	3.58±2.54	ND	17.18±1.97	75.88±1.97	0.67±0.06	21.4	113.2
Vigna unguiculata (L.) Walp.	0.93 ± 0.17	39.46±1.19	5.02±0.67	8.08±0.38	29.91±0.80	16.04±0.07	1.0	1.9

Fat content and fatty acid composition are expressed in percentage. Numerical data from each column is expressed as average ± standard deviation (n=3) PUFA/SFA: the ratio polyunsaturated fatty acids to saturated fatty acids, ω6/ω3: the ratioomega-6 fatty acids to omega-3 fatty acids. ND: not detectable

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