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B-CAROTENE CONTENT OF *M. LONGIFOLIA* SEED OIL IN DIFFERENT AGRO-CLIMATIC ZONES IN SRI LANKA, THE EFFECT OF HEAT ON ITS STABILITY AND THE COMPOSITION OF SEED CAKE

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ABSTRACT

M. longifolia is a plant with a seed rich of edible oil (more than 50%), but is still under-utilized for edible purposes in Sri Lankan context. It shows a wide distribution throughout the country representing several agro-climatic zones. No studies have done yet to discover variations of *M. longifolia* seed oil with respect to its different geographical locations. In this study, the content of β-carotene in *M. longifolia* seed oil samples obtained from four different agro-climatic zones in Sri Lanka was evaluated. The effect of heat on the stability of β-carotene in *M. longifolia* seed oil was also studied. Dried, fallen seeds were collected from randomly selected trees in four agro-climatic zones in Sri Lanka named low country dry zone (LD), low country wet zone (LW), low country intermediate zone (LI) and mid country intermediate zone (MI). Oil was extracted with a small scale, mechanical oil expeller (cold pressed method). β-carotene content in four samples was assessed with MPOB test method using Ultraviolet-Visible (UV-VIS) spectrophotometer and with High Performance Liquid Chromatography (HPLC) using Chase et al., (1994) method. A series of heat treatment (50 °C – 300 °C) was given and the content of β-carotene was determined at each temperature with the above mentioned two methods. There were some differences in the content of β-carotene for two types of methods. β-carotene content varies from 17.69 to 13.51 ppm in four agro-climatic zones for HPLC method and 20.46 – 27.69 ppm for spectrophotometric method. The reduction of β-carotene content up to 150 °C from the room temperature (30 °C) was not prominent. But after 150 °C, a sudden, sharp decrease was reported. Nutritional composition of seed cake varied significantly (p <0.05) among the different agro-climatic zones. Protein content, similar to Palm kernel was reported which ranged from 15.44 – 17.76%.

Keywords: *Madhuca longifolia*; β-carotene; heat stability

INTRODUCTION

M. longifolia is a large grown, woody tree of family Sapotaceae and reported to have its origin in Southeast Asia. This plant plays a significant role in both Indian and Sri Lankan traditional ayurvedic medicines. Almost all the parts of the plant including bark, flowers, seeds and leaves are used for that. The strong, hard and durable heartwood of the tree is used for house construction like activities. Flowers are edible and used as a food item of tribal either in raw or cooked forms. They are fermented to produce an alcoholic drink called as Mahua, country liquor. This liquor is used to produce vinegar also.

M. longifolia plant produces fruit which is economically valued for its seed which yield high quantity of fat (ca. 50%), commercially known as mahua butter or mowrah butter, and has many edible and medicinal applications **(Singh and Singh, 1991)**. It is one of the single largest sources of natural hard fat **(Bringi, 1987)**. This fat is used as a substitute for cocoa butter and ghee. Other than that, it has applications in cooking, frying and manufacturing chocolates. The seed fat has emuluscent property. Besides edible and medicinal uses, mahua has industrial application as it can be utilized in the manufacture of laundry soaps and lubricants **(Parrota, 2001)**.

The oil yield from the crops is always the key factor to decide its suitability for nutritional and industrial purposes from economic point of view **(Yadav et al., 2011)**. In that case, *M. longifolia* yields a considerable amount of fat as mentioned previously as well as it has a beneficial fatty acid profile with less saturated fatty acid content. In *M. longifolia* seed fat, 46% of the fatty acids present are saturated, 37.4% are monounsaturated, and 16.5% are polyunsaturated **(Ramadan et al., 2006)**. But the use of this oil for the food industry has been carried out in a limited scale. In Sri Lanka, almost all these edible purposes have limited only for cooking in traditional culture. This also is in very small scale and considers that seeds as an under-utilized seed type for the production of oil. According to the past reports, the under-utility of this fat may probably due to the lack of technical information regarding to the properties and potential uses.

Even though few reports in previous literature highlight several compositional characteristics and thermal properties of *M. longifolia* seed fat, no details can be found with the available pigments in it. The bright yellow color of *M. longifolia* seed fat gives evidences for that it contains considerable amount of pigments, especially β-carotene. Carotenoids are the phytonutrients that impart a distinctive yellow, orange, and red color to various plant parts. Among the carotenoids, β-carotene is important for its associated health benefits more than its color imparts for food stuffs. It is the most potent precursor of vitamin A and is present naturally as a mixture of various isomers (cis and trans) of β-carotene molecule. It has a potent

antioxidant capacity and offers an array of health benefit such as lowering the risk of heart diseases and certain types of cancers, enhancing the immune system, and protection from age-related macular degeneration; the leading cause of irreversible blindness among adults. Consumer attitude towards bioactive compounds, including β-carotene, as natural colorants and for health benefits is promising **(Gul et al., 2015)**. Therefore, identifying novel sources of β-carotene which acts as primary or only source of vitamin A in several countries is important.

After the extraction of oil from oil bearing seeds, a major portion of the raw materials is left over as the oil seed cake. Oil cakes are of two types, edible and non-edible **(Ramachandran et al., 2007)**. Edible oil cakes have a high nutritional value; especially have protein content ranging from 15% to 50% (www.seaofindia.com). Due to their rich protein content, they are used as animal feed, especially for ruminants and fish. Their composition varies depending on their variety, growing condition and extraction methods **(Ramachandran et al., 2007)**. The market value of the cakes is governed by its protein contents and quality of its proteins **(Kureel et al, 2009)**. Therefore, the determination of proximate composition of *M. longifolia* seed cake will beneficial to identify and enhance its potential uses.

The objective of this study was to determine the β-carotene content in *M. longifolia* seed oil grown in different agro-climatic zones in Sri Lanka and to assess the stability of β-carotene prior to any heat treatment and after a series of heat treatments. Other than that, the proximate composition of seed cake was also analyzed based on their different growing locations.

MATERIAL AND METHODOLOGY

Seeds Sample collection

The dried, fallen fruit seeds of *M. longifolia* were collected from four different agro-climatic zones in Sri Lanka during August-December 2014 using the random sampling method. Even though there are seven agro-climatic zones in Sri Lanka, these four were selected for the study based on the availability of plant. The four agro-climatic zones were low country dry zone (LD), low country wet zone (LW), low country intermediate zone (LI) and mid country intermediate zone (MI). The collected samples were named with symbols as LD, LW, LI and MI with respect to their agro-climatic zone. The species and the variety of the plant were confirmed by comparing the morphological characteristics.

Oil extraction

The seed coat was broken by hand and the kernels were removed out. Thereafter, kernels were ground to obtain a power using a domestic grinder. That powdered kernel was put into a small scale mechanical oil expeller and the oil was extracted (cold pressed method). The extracted oil was covered with Black papers and stored in a refrigerator (4 °C) for the determination of β-carotene content.

Heat treatment

The oil stored at refrigerator was taken out and allowed to come to the room temperature (30 °C). Then about 2 – 3 g of oil at room temperature was kept as it is to determine the β-carotene content at room temperature. All four samples at room temperature obtained from different agro-climatic zones were used to determine and compare the β-carotene content. After that, only the oil sample obtained from low country dry zone (LD) was heated to 50 °C, 70 °C, 100 °C, 150 °C, 200 °C, 250 °C and 300 °C to determine the stability of β-carotene. At each temperature, 2 – 3 g of oil was taken and stored for the determination of β-carotene content at those temperatures.

Determination of β-carotene content

Spectrophotometric determination was done by Ultraviolet-Visible (UV-VIS) spectrophotometer (UV mini 1240 ,SHIMADZU, Japan) at 450 nm using MPOB test method.

The sample was homogenized and weighed to the nearest ±0.0001 g into a 25 mL volumetric flask. The sample was dissolved with *n*-hexane and diluted to the mark. The solution was transferred into a 1 cm quartz cuvette and the absorbance was measured at 450 nm against *n*-Hexane. The carotene content was calculated as β-carotene in parts per million (ppm).

The calculation was as follows:

$$\beta\text{-carotene content (ppm)} = [V \times 383 \times (A_s - A_b)] / (100 \times W)$$

Where:
V = the volume used for analysis
383 = the extinction coefficient for carotenoids
A_s = the absorbance of the sample
A_b = the cuvette error
W = the weight of the sample in g

HPLC analysis was performed according to the method of **Chase et al., (1994)**.

About 1 g of sample was measured to a 25 mL volumetric flask with the 0.0001g accuracy. The content of the flask was filled with *n*-Hexane, mixed until dissolved and left for 12 hours at dark. Thereafter, it was centrifuged at 500 rpm in a laboratory centrifuge. A standard β-carotene sample was purchased from Merck and solution of β-carotene standard (5 mg/100 mL) was freshly prepared in *n*-Hexane and stored in the dark. This solution was further diluted with *n*-Hexane to the final concentration of 1 µg/mL. HPLC determination was performed with HPLC, Agilent technologies, Column = 4.6 x 250 mm Eclipse Plus C_{18} (5 µm), injector volume = 50 µL, Mobile phase = Acetonitrile: Ethyl Acetate: Methanol, 70:20:10, Flow rate = 1 mL/min, open temperature = 30 °C, UV-VIS detector, λ = 450 nm. Peaks were identified by comparing the retention times for the samples of oil and standard solution of known concentration of β-carotene. The quantity of β-carotene was expressed as parts per million (ppm) by comparing the area of peaks after separation of the β-carotene standard and oil samples. All samples were prepared in duplicate and the analysis at each temperature was done twice.

Determination of proximate composition

Powdwred M. Longifolia seed kemel was obtained by grinding the kamel. Moisture content was determinated according to A. O. A. C. method 925.13. Ash content was determined with A. O. A. C. method 923.03 - direct method. Protein and Fibre contents were analyzed with A. O. A. C. official method 984.13 and A. O. A. C. method 1985.

RESULTS AND DISCUSSION

β-carotene content and its stability

Table 1 indicates the β-carotene content (ppm) of *M. longifolia* seed oil (determined by two methods) obtained from four different agro-climatic zones in Sri Lanka. According to the results, β-carotene content varies from 13.51 to 17.69 ppm in four agro-climatic zones for HPLC method. The highest β-carotene content has reported in the sample obtained from low country dry zone (LD). The second highest content was there in the mid country intermediate zone (MI) sample. Under the spectrophotometric method, the results were somewhat higher than the HPLC. Here also, the sequence of β-carotene content from highest to least is similar to the results of HPLC method. The β-carotene content was ranged from 20.46 – 27.69 ppm by spectrophotometric method. Fig. 1 compares the value determined by two different methods. The differences in the sensitivity of the two methods may be the reason for the variations obtained under two methods in determining β-carotene content.

According to the previous studies, the quantity of β-carotene in other vegetable oils determined by spectrophotometric method is 542.09 ppm for Red palm olein, 0.00 ppm for palm olein, 0.91 ppm for corn oil and 0.00 ppm for coconut oil **(Dauqan *et al.*, 2011)**. The literature regarding the other vegetable oil types is difficult to find. Above results shows that except Red palm olein, other oil types are lack of β-carotene. However since these oils were commercially available oils, it is difficult to get an idea about the naturally available content of β-carotene. During processing, a significant amount can be lost because the stability of β-carotene depends on light, heat etc. like external factors. When compared with above oils, β-carotene content in *M. longifolia* seed oil is considerable (20.46 – 27.69 ppm, determined by spectrophotometric method).

Table 1 β-carotene content (ppm) determined by two different methods at room temperature (30 °C)

Sample code	β-carotene content (ppm, HPLC method)	β-carotene content (ppm, spectrophotometric method)
LD	17.69 ±0.23	27.69 ±0.00
LW	13.51 ±0.31	20.46 ±0.32
LI	14.32 ±0.05	22.67 ±0.10
MI	17.52 ±0.11	27.42 ±0.00

Note: Results are Mean ±Standard Deviation (*n=3*).

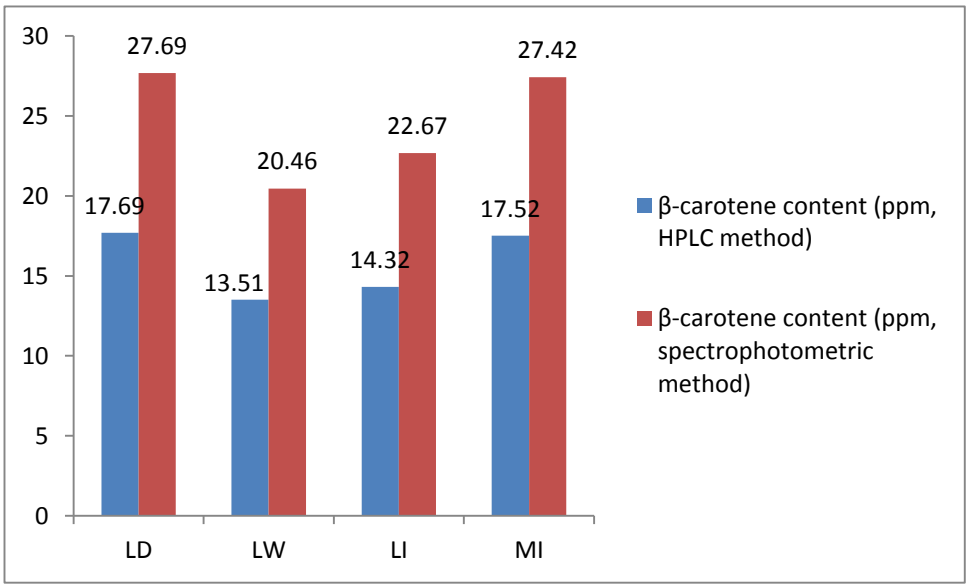

Figure 1 β-carotene content (ppm) of *M. longifolia* seed oil in four agro-climatic zones.

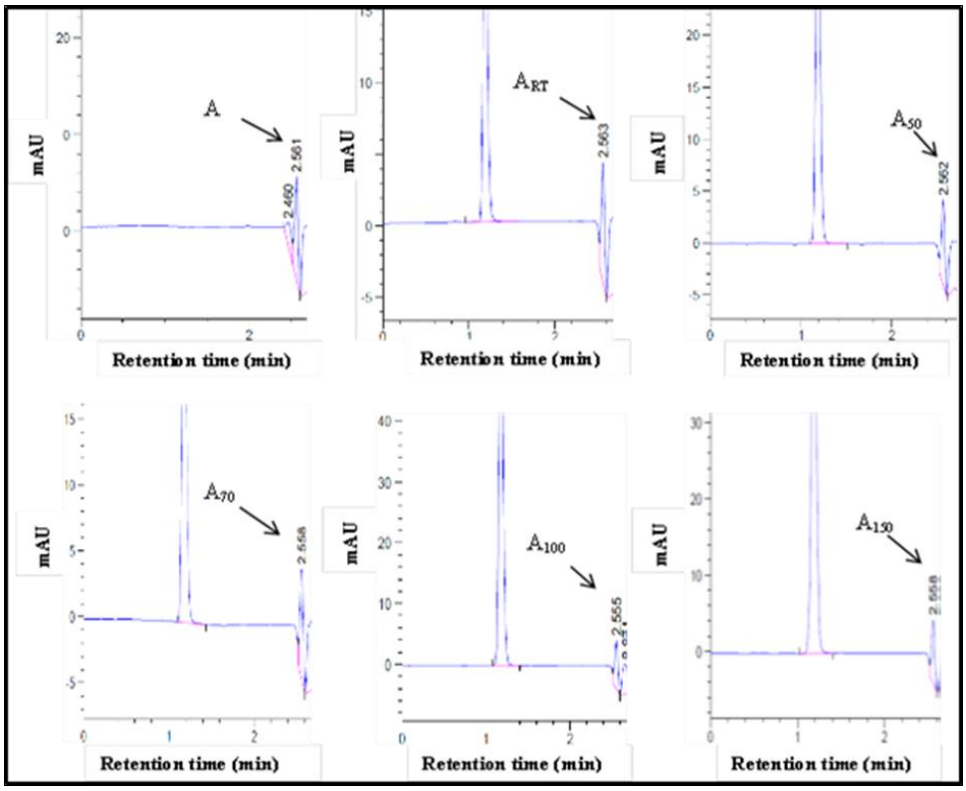

Figure 2 Peaks for β-carotene in HPLC chromatogram for standard (A), at room Temperature (A_{RT}), at 50 °C (A_{50}), at 70 °C (A_{70}), at 100 °C (A_{100}) and at 150 °C (A_{150}).

Table 2 β-carotene content (ppm) determined by two different methods at different temperatures.

Temperature (° C)	β-carotene content (ppm, HPLC method)	β-carotene content (ppm, spectrophotometric method)
50	17.70 ±0.00	26.53 ±0.00
70	17.48 ±0.20	25.64 ±0.00
100	17.47 ±0.14	22.82 ±0.01
150	17.25 ±0.27	21.31 ±0.32
200	0.00	9.41 ±0.19
250	0.00	4.13 ±0.00
300	0.00	1.45 ±0.00

Note: Results are Mean±Standard Deviation (n=3).

The oil sample obtained from low country dry zone (LD) was used to study the effect of heat treatment on β-carotene content. Here, a prolonged heat treatment was not given and only the stability of β-carotene at higher temperatures was evaluated. Fig. 2 illustrates how the peaks in HPLC chromatogram were obtained for the standard sample of β-carotene and for the other samples of *M. longifolia* seed oil which were subjected to the heat treatment. According to the results of Table 2 for HPLC method, the reduction of β-carotene content up to 150° C from the room temperature was not prominent as it has decreased from 17.70 ppm to 17.25 ±0.27 ppm only. But after 150 °C, a sudden, sharp decrease (almost zero value) was observed as shown in Fig.3. This pattern of behavior was common for the spectrophotometric method also. By the way, even after 150° C also, small quantity of β-carotene was detected by spectrophotometric method in contrast to HPLC method.

In many parts of the less industrialized world, vitamin A deficiency is a problem that affects nutritional status and health. In several countries, provitamin A carotenoids are the primary, if not the only, source of vitamin A **(Oliveira *et al.*, 1998)**. For humans, β-carotene of vegetables is considered as an important source of vitamin A. Among the vegetable sources, vegetable oils is a suitable carrier of β-carotene since it is a daily fat source, high in energy, polyunsaturated fatty acids, and naturally occurring antioxidant vitamin E. Therefore, vegetable oils like *M. longifolia* seed oil which is rich with β-carotene would be beneficial in human nutrition since it is stable even at higher temperatures like 150 °C.

Proximate composition of seed cake

When consider about the parameters mentioned in Table 3, significant differences (p <0.05) were there in all the analyzed parameters for different agro-climatic zones. Moisture content was ranged from 9.5 – 10.86%, protein from 15.44 –17.76%, ash from 1.62 – 2.44% and fibre from 28.03 – 30.59%. In oil seeds, protein content is the most valuable parameter which determines its potential applications. The content of protein of widely available oil seeds are as follows.

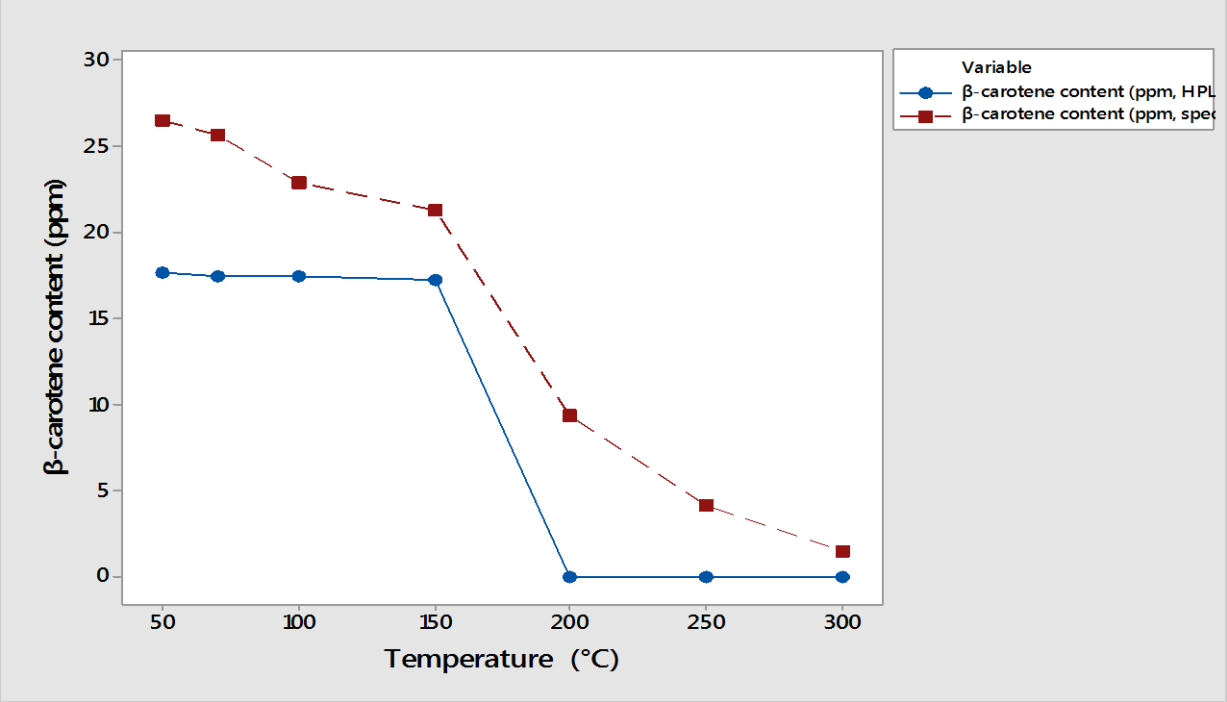

Figure 3 Stability of β-carotene at higher temperatures in *M. longifolia* seed oil.

Table 3 Proximate composition of seed cake in different agro-climatic zones

Parameter	LD	LW	LI	MI
Moisture (%)	9.52 ±0.46^{c}	10.09 ±0.21^{b}	10.29 ±0.25^{b}	10.86 ±0.32^{a}
Protein (%)	15.44 ±0.43^{b}	15.72 ±0.34^{b}	15.87 ±0.60^{b}	17.76 ±0.46^{a}
Ash (%)	2.06 ±0.10^{b}	2.44 ±0.22^{a}	1.67 ±0.05^{c}	1.62 ±0.03^{c}
Fibre (%)	30.09 ±0.75^{a}	28.03 ±0.51^{b}	28.09 ±0.33^{b}	30.59 ±0.38^{a}

Note: Reults are Mean ±Standard Deviation (*n=3*),
Means that do not share a letter are significantly different at significant level 0.05 (Fisher LSD test).

For Coconut, Cotton seed, Ground nut, Olive, Palm kernel, Soy bean and Sunflower, values are 25.2%, 40.3%, 49.5%, 6.3%, 18.6%, 47.5% and 34.1%. With them, *M. longifolia* seed cake has a value for protein similar to Palm kernel (18.6%). Location wise differences showed that, samples from mid country intermediate zone has the highest protein content (17.76%) compared to other three zones. Fibre values for above mentioned seed cakes are as 10.8%, 15.7%, 5.3%, 40%, 37%, 5.1%, and 13.1% for Coconut, Cotton seed, Ground nut, Olive, Palm kernel, Soy bean and Sunflower respectively. Fibre value of *M. longifolia* seed cake is also (28.03 – 30.59) somewhat closer to that of Palm kernel (37%).

CONCLUSION

β-carotene content in four agro-climatic zones varies from 13.51 to 17.69 ppm by HPLC method and 20.46 – 27.69 ppm by spectrophotometric method. The loss of β-carotene is not prominent up to 150° C and thereafter, a sharp decrease can be seen. When compared with the commercially available other vegetable oils, *M. longifolia* seed oil is rich with β-carotene. *M. longifolia* seed cake is composed of a considerable amount of protein comparable to other commonly available oil seed cakes and therefore it can be utilized as a source of protein in food applications.

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