

**PROXIMATE CONTENT AND LIPID PROFILE OF SEEDS FROM *RAPANEA MELANOPHLOEOS* (THE CAPE BEECH) TREE**

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Running title: composition of *R. melanophloeos* seeds

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## **Abstract**

*Rapanea melanophloeos* (Cape beech) is a fruit-bearing tree indigenous to Southern Africa which produces edible fruit. Previous studies have focussed on investigating the nutritional potential of the tree's fruit pulp. The nutritional potential of *R. melanophloeos* seeds is unknown. Seed samples obtained from ripe fruit of the Cape beech trees had their proximate analysis and lipid profiling done. The dry matter and ash contributed  $91.29 \pm 0.00$  %,  $1.50 \pm 0.01$  % of the mass of the seed. Whilst the other proximate analytes namely crude fibre, crude protein and ether extract made up  $5.71 \pm 0.43$  % ,  $10.50 \pm 0.49$  % and  $4.75 \pm 0.09$  % the mass of the seeds respectively. The fatty acid profile of the seed oil revealed Linoleic acid ( $50.43 \pm 0.38$  %) to be the most dominant. *R. melanophloeos* seeds are not a viable source of nutrients.

## **Introduction**

There is a limited availability of non-renewable energy sources, whilst the use and search for the non-renewable energy sources poses serious threats to the environment (Demirbas & Demirbas, 2011). Bio-renewables are clean and environmentally friendly alternative sources of energy (Dincer, 2008). Plant derived oils constitute an important renewable resource (King *et al.*, 2009) for the chemical, cosmetics and pharmaceutical industries. Most plant seeds are reservoirs of large quantities of oils with fatty acid profiles (Thelen & Ohlrogge, 2002) that are potentially useful energy or nutrient sources. Plant seeds are also rich sources of protein for human consumption and as additives for livestock feed production (Van Etten, 1967). There is an abundance of indigenous fruit-bearing trees in Africa, whose seeds are often discarded and not exploited for their often rich nutrient and seed oil content.

We have previously shown that the seeds of some indigenous fruit bearing trees such as *Kigelia africana* and *Terminalia sericea* are macro-nutrient dense (Chivandi *et al.*, 2008;

Chivandi *et al.*, 2013) and can thus serve as alternative sources of nutrition for both humans and animals.

*Rapanea melanophloeos*, is commonly called the Cape beech tree and has a broad distribution in the forests of Southern Africa, from Cape Town in the south to Zambia in the north (Ohtani *et al.*, 1993; Van Wyk & Van Wyk, 1997). It is a dense, ever-green tree of about 4-10 meters in height (van Wyk & van Wyk, 1997). It has dark green leathery leaves with distinct purplish petioles (Moll, 1967). Its scented flowers usually appear in clusters along the branches on knobs (Palmer & Pitman, 1961). The tree's fruits are eaten by insects, birds, monkeys and wild pigs (van Wyk & van Wyk, 1997).

In Southern and Eastern Africa, the different parts of *R. melanophloeos* are widely used as ethnomedicines for many ailments (Ohtani *et al.*, 1993). Decoctions of the bark and fruits are used therapeutically for stomach ailments, respiratory and skin disorders (van Wyk & van Wyk, 1997). Bark and root extracts or decoctions are also used in the treatment of fever, diabetes mellitus, stomach and muscular pain (Pujol, 1990; van Wyk & van Wyk, 1997). In some communities, bark decoctions are used as expectorants, emetics and enemas (Watt & Breyer-Brandwijk, 1962). In ethno-veterinary medicine, *R. melanophloeos* is used as an anti-helminthic (Dold & Cocks, 2002). Ohtani *et al.*, (1993) reported on the anti-molluscicidal and anti-fungal activity of the triterpenoid saponins from the leaves of *R. melanophloeos*. Extracts from the stem bark have been shown to have *in vitro* antiplatelet aggregation activity (Mosa *et al.*, 2011). The research that has been done on *R. melanophloeos* has mainly focussed on its ethnomedicinal uses without exploring its seed's potential yield of oils and macronutrients. Thus, this study aimed to ascertain the proximate content and lipid profile of the seed of *R. melanophloeos* to investigate its feasibility for use as a nutritional supplement and a source of oil for pharmaceutical, cosmetics and chemical industries.

## **Materials and methods**

### **Seed source**

The *R. melanophloeos* fruits (Figure 1a) were harvested from trees in the medicinal botanical display garden at the Faculty of Health Sciences Parktown campus of the University of the Witwatersrand (coordinates: 26° 10' 52.96" S, 28° 2' 33.61" E) in April 2015.

**Figure 1a, b and c. Photographs showing *R. melanophloeos* fresh fruits (A) dry ripe fruits (B) and seeds with fruit pulp removed (C).**

### **Processing of the seeds**

The ripe fruits were placed in an oven set at 30°C to dry for 48 hours. The seeds were then manually extricated and cleaned of the desiccated fruit pulp (Figure 1b and 1c). The seeds were crushed manually into a fine powder using an electric blender (Waring, Lasec Pty Ltd., Johannesburg, South Africa) and then kept in a dark sealed container (Chivandi *et al.*, 2011a) pending assays.

### **Proximate analysis**

The proximate analyses were undertaken at an accredited laboratory (Agricultural Research Council-ARC) in Irene South Africa. Dry matter determination was undertaken according to the Official Methods of Analysis of Analytical Chemists (AOAC, 2005). The protein, ash, crude

fibre and, ether extract of the seed meal were also quantified using standard protocols (AOAC, 1995).

### **Seed oil lipid profile**

The lipid profile to quantify the fatty acids in the seed oil was also undertaken at the ARC Analytical laboratory In Irene, South Africa following the protocol of Christopherson and Glass (1969). To separate fatty acids, an OmniSper 5 C18 150 × 4.6 analytical column and guard-column were used. An external calibration method was used for quantification. The protocol and equipment used have been described fully in our previous work on seed oils (Chivandi *et al.*, 2013).

### **Results**

Table 1 shows the quantity of the proximate analytes of the Cape beech seeds. Whilst the fatty acid profile of the oil extracted from the seeds of *R. melanophloeos* shown in Table 2. The crude protein content was  $10 \pm 0.49\%$  while the seed oil content was  $4.75 \pm 0.09\%$ . *R. melanophloeos* profiling of the oil showed the presence of saturated ( $19.54 \pm 0.50\%$ ) fatty acids, monounsaturated ( $37.27 \pm 0.56\%$ ) and polyunsaturates ( $51.77 \pm 0.22\%$ ) were more abundant. The dominant saturated fatty acids were palmitic ( $14.72 \pm 0.09\%$ ) and stearic ( $3.02 \pm 0.05\%$ ) acid, whereas oleic ( $36.49 \pm 0.35\%$ ) and linoleic acid ( $50.43 \pm 0.38\%$ ) were the major unsaturated fatty acids.

### **Discussion**

The seeds of *R. melanophloeos* had a low yield of protein (approximately 11%) when compared to crude protein content of the common plants used as protein sources used in the formulation of animal feeds, for example sunflower seeds (20-24 % CP) or soybeans (approximately 45%

CP) (Iqbal *et al.*, 2006). However, it compares well with that of ordinary maize varieties (8-11%; crude protein) (FAO, 1992). The seeds of *R. melanophloeos* are therefore not suitable for use as sole protein sources in either human or monogastric animal diets.

When seeds have a high oil yield (ether extract) they could be exploited as possible commercial sources of plant-derived oil. Several factors influence the amount of oil extracted and the composition of seed oil. Some of the factors are the extent to which the seeds have matured (Al-Khalifa, 1996), as well as the ambient climatic conditions and geographic location the plant (Salimon & Abdullah, 2008). The process used to extract the oil also impacts on the yield and nature of the oil (Mitra *et al.*, 2009). At  $4.75 \pm 0.09$  %, the *R. melanophloeos* seeds oil content was almost a tenth that sunflower seed (35-40 %) (Chivandi *et al.*, 2013) which is commonly used as an edible oil. The oil yield was also low when compared to that of other indigenous fruit bearing trees from southern Africa such as *Mimusops zeyheri* (212. 50g.kg<sup>-1</sup> DM; 28%) (Chivandi *et al.*, 2011a) and *Kigelia africana* (492. 20g.kg<sup>-1</sup>, 51%) (Chivandi *et al.*, 2011b).

The seed oil of the Cape beech consisted predominantly of polyunsaturated fatty acids (52%) whilst the saturated fatty acids comprised 9.5%. Interestingly, 51 % of the oil was unsaturated *cis* fatty acids. The *cis* fatty acids such as Oleic acid (97% of monounsaturated fatty acids in *R. melanophloeos*) and Oleanolic acid (97% of polyunsaturated fatty acids in *R. melanophloeos*) are beneficial to cardiovascular health as their intake promotes the *de novo* synthesis of high density lipoproteins (HDLs) that reduce the risk of heart disease (Zock & Katan, 1992). The preponderance of the essential monounsaturated omega 9 and polyunsaturated omega 6 fatty acids could be exploited to improve the quality of life of consumers as these essential fatty acids (EFAs) have beneficial effects on cardiovascular health and are known to reduce inflammation (Simopoulos, 2002). Further analysis of the omega 6 to omega 9 fatty acid ratio ( $1.38 \pm 0.60$ ) of the seed oil, showed that it was low thus potentially making the oil beneficial in the suppression of cardiovascular and inflammatory diseases (Simopoulos, 2002). Unfortunately, despite being rich in omega 6, omega 9 and *cis* fatty acids, the high ratio of

omega 6 to omega 3 fatty acids (40%), indicated that the seed would exacerbate inflammatory processes and cardiovascular disorders (Simopoulos, 2002) if ingested in large amounts. The relatively high amounts of linoleic acid in the *R. melanophleos* oil make it a suitable candidate for the essential fatty acids, for use in edible oils (Nyam *et al.*, 2009) if the yields were high enough to be economically exploited. Oleic acid (which the seed oil also contained), when incorporated into diets at high amounts, has been reported to reduce the risk of developing hypertension and also decreases blood pressure (Alonso & Martínez-González, 2004).

Dermatologically, Oleic acid is exploited as an enhancer for absorption percutaneously (Pathan and Setty, 2009). The essential fatty acid, Linoleic acid plays a major role in maintaining the health and structure of skin. When linoleic acid is deficient, dermatological problems and alopecia occur (Cunnane & Anderson, 1997). The Cape Beech seed oil thus has potential to be exploited to make skin emollients and ointments for percutaneous delivery of pharmaceutical agents as well as for skin moisturisers. Palmitic acid and stearic acid were the predominant non-essential fatty acids isolated from the seed oil. These two fatty acids are mainly used in the formulation of cosmetics, soaps and creams used for shaving (Dangarembizi *et al.*, 2015).

### **Conclusion**

Although the lipid profile of the oil from seeds of the Cape Beech oil contained physiologically and economically important saturated and mono- and poly- unsaturated fatty acids, the oil content and crude protein content was too low to make it a viable alternative bio-renewable resource for energy and or nutrients.

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## Tables

**Table 1:** Proximate composition of *R. melanophloeos* seeds.

	%
Dry matter	91.29 ± 0.00
Ash	1.50 ± 0.01
Crude protein	10.50 ± 0.49
Ether extract	4.75 ± 0.09
Crude fibre	5.71 ± 0.43

Data expressed as mean ± standard deviation after analysis (triplicate).

**Table 2:** Lipid (Fatty acid) profile of *R. melanophloeos* seed oil.

<b>Fatty acid</b>		<b>%</b>
Palmitic acid	C16:0	14.72 ± 0.09
Stearic acid	C18:0	3.02 ± 0.05
Arachidic acid	C20:0	0.55 ± 0.02
Behenic acid	C22:0	0.69 ± 0.59
<b>TSFA</b>		19.54 ± 0.50
Oleic acid	C18:1n9c	36.49 ± 0.35
<b>TMUFA</b>		37.27 ± 0.56
Linoleic acid	C18:2n6c	50.43 ± 0.38
α-linoleic acid	C18:3n3	0.50 ± 0.38
<b>TPUFA</b>		51.77 ± 0.22
Trans fatty acids		0.13 ± 0.04
Cis fatty acids		86.91 ± 0.70
Omega 3		1.26 ± 0.60
Omega 6		50.57 ± 0.39
EPA		0.43 ± 0.30
Omega 9		36.66 ± 0.32

Omega 6: Omega 3		40.13 ± 0.60
Omega 6: Omega 9		1.38 ± 0.60

Data expressed as mean ± standard deviation after analysis (triplicate).

TSFA: Total saturated fatty acids; TMUFA: Total monounsaturated fatty acids; TPUFA: Total polyunsaturated fatty acids.



**Figure 1a, b and c. Photographs showing *R. melanophloeos* fresh fruits (A) dry ripe fruits (B) and seeds with fruit pulp removed (C).**