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QUERCUS ROBUR (ENGLISH OAK) SEED: A POTENTIAL ENERGY, OLEIC AND CIS-LINOLEIC ACID RICH NUTRITIONAL SUPPLEMENT IN SOUTH AFRICA

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Abstract

Although plentiful in the South African urban areas where it is used as an ornamental tree, *Quercus robur* (*Q. robur;* English Oak), is not indigenous. The tree produces an abundance of fruit (acorns) which contain seeds that are left to decay despite their potential as sources of biomass. To establish the potential of the tree, we determined the proximate, mineral, fiber, amino acid, fatty acid and phytate-phosphate content of dehulled *Quercus robur* seed. The seed had a dry matter (DM) content of 91.56% and a gross energy content of 17.38 MJ kg^{-1}. Of the DM content 88.65% was organic matter, 8.77% crude protein (CP) and 4.55% lipid. Calcium (0.07%) and phosphorus (0.11%) concentration were low. Amino acids constituted 94.86% of the CP. Saturated fatty acids (SFAs) constituted 18.18% of the lipid content with palmitic acid the dominant SFA while the monounsaturated fatty acids (MUFAs) made up 42.04%. Oleic acid (OA) constituted 97.66% of the MUFAs. Polyunsaturated fatty acids made up 30.22% of the lipid content with cis-linoleic acid (28.71%) dominating. Due to the high energy content, a preponderance of OA and cis-linoleic acid and a low phytate-phosphate and fiber content, *Q. robur* seed could potentially be exploited as an energy dense and healthful nutritional supplement.

Key words: *Quercus robur* seed, Chemical composition, Oleic acid, Linoleic acid, Nutritive potential.

Introduction

The oak tree is renowned in the annals of world literature as a massive, solid tree with a mutliplicity of uses. It belongs to the family Fagaceae; genus *Quercus*; and has over 500 species native to the temperate zones (Bonner & Vozzo, 1987). Oak trees which start to produce acorns when they are about twenty-five years old (Harper and White, 1974) are dicotyledonous, monoecious wind pollinated trees. The English oak, *Quercus robur*, is one of the British trees best known for its hardwood, sturdiness and long lifespan (Logan, 2005; Watson, 1948). *Q. robur* is deciduous and flowers in spring (Tansley, 1952). Its leaves are simple, elliptic to obovate with wavy margins divided into 3 to 6 round lobes and rounded at the tip (Russel *et al.,* 2007; Henderson, 2001). Leaf length and width ranges from 6 to 12 cm and 4 to 7 cm, respectively (Russel *et al.,* 2007; Henderson, 2001). *Q. robur* fruits, commonly called acorns, contain one seed; the nut. The acorn is the cup shaped circle of bracts about a third of the length of fruit enclosing the base of the nut.

Quercus robur seeds serve as a food source to invertebrates including wasps and weevils and vertebrates such as squirrels, wood mice and rabbits (Crawley & Long, 1995). Historically Oak acorns are reported to have supplied 25% of the food basket of the poor in the Mediterranean region (Hill, 1937). The use of Oak acorns to prepare foods or food ingredients that provide health benefits (functional foods) over and beyond supply of the basic nutrients is well documented (Rakić *et al*., 2004; Cantos *et al*., 2003; Lee *et al*., 1992). Phytochemicals inclusive of tannins, gallic, ellagic, and derivatives of galloyl and hexahydroxydiphenol contribute to the "functional food" status of the *Q. robur* acorns (Cantos *et al*., 2003). The presence of these phytochemicals accounts for the biological activities, such as antioxidant activity of the *Q. robur* acorns (Cantos *et al*., 2003; Rakić *et al*., 2004). Anti-nutritional factors, which when Oak acorns are consumed in large amounts negatively affect animal health, have been reported (De Boer & Bickel 1988). The practice of leaching the acorns before using them as feeds for livestock and human consumption are traditional attempts to render the acorns safer when consumed in large quantities (Fernand & Kinsey, 1943).

The English Oak, exotic to South Africa, was introduced in the country as early as the seventeenth century (Campbell & Moll, 1977; Kemp, 2002). The tree is found lining streets in cities such as Johannesburg, Stellenbosch, Sasolburg and Cape Town. Stellenbosch is reputed as the "Oak Tree City" of South Africa (Esterhuyse *et al*., 2001). Historical records indicate that in the 1880s when Afrikaner farmers settled on the Witwatersrand there were not many trees in sight. Johannesburg had typical savanna grassland with scattered scrubs and no naturally occurring trees (Esterhuyse *et al*., 2001). The early Afrikaner farmers came in with seeds of Oak and Walnut trees from the Cape region of South Africa and planted them in various regions of Johannesburg (Kemp, 2002). The Oak is common today as an ornamental tree in gardens, parks and avenues. Unlike the many thorny Acacia species which were indigenous to Gauteng, the thorn-free Oak was more favoured by the settlers as it posed no danger to vehicle and human traffic (Esterhuyse *et al*., 2001; Kemp, 2002).

The study sought to evaluate the nutritional potential of the *Quercus robur* found in Johannesburg, South Africa, by determining its seed's proximate, mineral, phytate-phosphate, fiber, amino acid and fatty acid composition.

Materials and Methods

Seed source and identification: Fresh ripe *Q. robur* fruits were collected from ten randomly selected *Q. robur* trees in

Braamfontein and Parktown suburbs, Johannesburg, South Africa [latitude 26° 10′ 0″ S, longitude: 28° 02' 0″ E] between February and March 2015. The area is located at 1750m above sea level with an annual rainfall range of 600 to 1400 mm and a mean annual maximum temperature of 26°C, respectively (Statistics South Africa, 2004). The fruits and leaves of the trees from which the fruit were collected were sent to the C.E. Moss Herbarium of the University of the Witwatersrand and authenticated as *Q. robur* with voucher number J86469 by Mr. Donald McCallum, a botanist in the herbarium.

Fruit processing and storage: The fruits were manually shelled and the seeds dried in the shade. The dried seeds were manually dehulled following which they were crushed using a blender (Waring; Lasec Pty Ltd, Johannesburg) to produce the seed meal. Figures 1 and 2 show the photographs of the dry *Q. robur* acorns and shelled seeds.

Chemical determinations: The Agricultural Research Council's Irene Analytical Services Laboratories, Pretoria, South Africa, assisted in the determination of the various chemical constituents of the *Q. robur* seed. Each of the assays was done in triplicate.

Determination of the proximate components: The dry matter, ash, crude protein content (CP), and the ether extract (EE), of the *Quercus robur* seed were determined as described by AOAC (2005; method numbers 934.01, 942.05, 954.01, and 920.39, respectively). The seed's gross energy value was determined using an MC-1000 Modular Calorimeter equipped with a desk top computer and MC1000 software.

Determination of the calcium and phosphorus content: The calcium and phosphorus content of the seed was determined as described by Zasoski and Burau (1977). In summary, 0.5 g of the seed meal sample was digested with concentrated nitric acid and perchloric acid at 200˚C to generate the digest solution from which an aliquot was used for the inductively coupled plasma optical emission spectrometric (ICP_OES) determination of calcium and phosphorus. The assay was then done on a Varian Liberty 200 spectrometer (Varian, Perth, Australia) as described by Huang and Schulte (1985).

Fig. 1 *Q. robur* acorns.

Fig. 2. Dehulled *Q. robur* seeds.

Determination of the fiber content: The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the method of Van Soest *et al.,* (1991). During the determination of NDF, about 0.5 g of the seed meal was refluxed for 1 h in 100 ml of neutral detergent solutions of sodium lauryl sulphate and ethylenediamine-tetra-acetic to which alpha-amylase (20 350 IU ml^{-1}) dietary fiber kit, Sigma-Aldrich, St Louis, USA) was added. The mixture was then filtered after refluxing for one hour, then dried and weighed. The ADF was determined by refluxing 0.5 g of the sample for 1h in acid detergent solution (20 g acetyl-trimethyl ammonium bromide dissolved in 1L normal H_2S0_4). The resulting mixture was then filtered and the residue dried and weighed.

Determination of the phytate-phosphate content: The phytate-phosphorus composition was determined calorimetrically using a Perkin Elmer Lambda25 UV/Vis Spectrometer equipped with a PC and Lambda25 software, as described by Wheeler and Ferrel (1971).

Determination of the amino acid content: The procedures described by Einarsson *et al.,* (1983) were used in determining the concentration of each of the assayed amino acids. In summary, the seed meal samples were hydrolysed with 6 M HCl at 110˚C for 24 h after which pre-column fluorescence derivatisation of amino acids was done by reacting them with 9-flourenylmethyl chloroformate. The amino acids were extracted using pentane and separated by gradient elution on a chromatograph. The chromatograph consisted of a SpectraSystem P4000 Quaternary high performance liquid chromatography (HPLC) equipped with a SpectraSystem FL 3000 fluorescence detector and Rheodyne 7125 valve with 20 uL injection pump. The buffer system used in the separation of the amino acids was varied from sodium citrate buffer (pH 2.95) of acetonitrile in the ratio of 70:30 to sodium citrate buffer (pH 4.5) of methanol-acetonitrile in the ratio of 14: 6: 70 and a flow rate of 1.4 mL min^{-1}. An OmniSper 5 C18 150 × 4.6 analytical column and guard-column were used in

the separation of the amino acids. Identification of the amino acids in the chromatogram was done at an excitation wavelength of 264 nm and an emission wavelength of 340nm (Einarsson *et al*., 1983*)*. A personal computer with TSP software was used to perform the quantification using an external calibration procedure.

Determination of the fatty acid content: The solvent extraction method using Soxhlet apparatus was used for fat extraction. The methylation of the lipid to produce methyl esters and the subsequent profiling and quantification of individual fatty acids was done as described by Christopherson & Glass (1969).

Data analysis: Each analyte's mean and standard deviation was computed from the triplicate assays. Data are given as mean ± SD.

Results

Table 1 shows the proximate, mineral, phytate-phosphate and fiber content of the seed. The seed had a dry matter content of 91.56% split as 88.65% organic matter, 8.77% crude protein (CP) and 4.55% lipid. It had a gross energy value of 17.38 MJ kg^{-1} and contained 0.07% and 0.11% calcium and phosphorus, respectively. The *Q. robur* seed meal phytate-phosphate, NDF, and ADF (0.07%, 24.76% and 3.61%, respectively) were low. Table 2 shows the amino acid profile of the seed meal. The amino acids constituted 94.86% of the CP with glutamic acid (1.02 g 100 g^{-1}) the most concentrated and tryptophan (0.03 g 100 g^{-1}) the least concentrated. Table 3 shows the fatty acid profile of the seed meal with five fatty acids (palmitic, oleic, stearic and the essential fatty acids (EFA) linoleic (C18:2n6c) and alpha-linolenic (C18:3n3) accounting for 88.05% of the fatty acid content. Saturated fatty acids (SFAs) constituted 18.18% of the lipid content with palmitic acid the dominant SFA while the monounsaturated fatty acids (MUFAs) made up 42.04% with oleic acid (OA) constituting 97.66% of the MUFAs. The polyunsaturated fatty acids (PUFAs) made up 30.22% of the lipid content with linoleic acid (18:2n6c) making up 95% of the PUFAs.

Table 1. Proximate, mineral, phytate-phosphate and fiber content of *Quercus robur* seed.

Proximate component	Mean +SD
Dry matter (g kg^{-1})	915.60 ± 0.30
Organic matter (g kg^{-1})	886.50 ± 0.03
Crude protein (g kg^{-1})	87.70 ± 0.31
Ether extract (g kg^{-1})	45.50 ± 0.03
Ash (g kg^{-1})	29.13 ± 0.30
Gross energy (MJ kg^{-1})	17.38 ± 0.07
Mineral	
Calcium (Ca^{2+}) (%)	0.07 ± 0.01
Phosphorus (P) (%)	0.11 ± 0.00
Phytate-phosphate (%)	0.07 ± 0.01
Fiber fraction (g kg^{-1})	
Neutral detergent fiber	247.59 ± 1.07
Acid detergent fiber	36.11 ± 0.22

Data are presented as mean ±SD, n = 3

Table 2. Amino acid content of *Quercus robur* seed.

Amino acid (g 100 g^{-1})	Mean ±SD
Glutamic acid	1.02 ± 0.38
Arginine	0.94 ± 0.28
Aspartic acid	0.86 ± 0.47
Serine	0.63 ± 0.33
Leucine	0.56 ± 0.05
Glycine	0.52 ± 0.12
Alanine	0.50 ± 0.12
Lysine	0.47 ± 0.03
Valine	0.41 ± 0.01
Proline	0.40 ± 0.09
Phenylalanine	0.38 ± 0.08
Histidine	0.37 ± 0.22
Isoleucine	0.35 ± 0.02
Threonine	0.35 ± 0.04
Tyrosine	0.23 ± 0.07
Cysteine	0.16 ± 0.02
Methionine	0.08 ± 0.02
Hydroxy-proline	0.06 ± 0.02
Tryptophan	0.03 ± 0.00
Total	**8.32**

Data presented as mean ± SD, n = 3

Discussion

Proximate, mineral and fiber composition: Rakić *et al.,* (2006) reported *Q. robur* acorn as containing 92.1% dry matter (DM), 4.8% CP, 0.1% calcium and 0.1% phosphorus. The DM, calcium and phosphorus of *Q. robur* as reported by Rakić *et al.,* (2006) is similar to that from *Q. robur* seed from Johannesburg. However, the CP content of *Q. robur* from Johannesburg, at 8.77%, was 83% higher compared to that reported by Rakić *et al.,* (2006) making the Johannesburg *Q. robur* provenance seed nutritionally superior. However, in a study of the protein content of twenty *Quercus* taxa in Turkey, Özcan, (2006) reported the CP content of mature acoms to range from 2.75 to 8.44% with the upper range in agreement with the findings of our current study. Although the CP content of the *Q. robur* seed meal from the Johannesburg provenances at 8.77% is lower compared to that of legume seeds and that of *Moringa oleifera* leaf meal (Makkar & Becker, 1997), it compared favourably with the 8 to 11% CP content of maize varieties (FAO, 1992) and 8.8% CP content of sorghum (Rakić *et al.,* 2006) . The CP content of *Q. robur* seed is within the 8.65 to 12.5% CP range of sorghum seed meal (Deyoe, & Shellenberger, 1965) which (sorghum seed meal) is an important cereal grain developing in tropical Africa. According to Notter *et al*., (2017) *Rapanea melanophloes* seed meal (from trees found within the same locality as the *Q. robur* used in the current) has a CP content of 1050% and a lipid content of 4.75%. Thus while the *Q. rubor* seed meal has a lower CP content compared to that of *R. melanophloes*, its lipid content (4.55%) is not significantly different to that of *R. melanophloes.* *Q.*

robur seed contained eight essential amino acids. In contrast to the *Q. robur* reported on by Özcan, (2006) the *Q. robur* seed from Johannesburg also contained the amino acids arginine, alanine, proline, cysteine and methionine. The *Q. robur* seed meal's gross energy content of 17.38 MJ kg^{-1} is comparable to the 17 MJ kg^{-1} reported for maize (Fagbenro, 1999). At 4.55%, the lipid content of *Q. robur* seed compares favourably the 4.4% lipid content of *Zea mays* (Watson, 2003) but was much lower than the 5-25% lipid content in soybean seeds (Cheftel & Cheftel, 1977). Maize is one of the major contributors to the dietary energy supply in most of sub-Saharan Africa. Thus *Q. robur* seed meal could potentially be used as an energy source in foods and feeds. When compared to the calcium and phosphorus content of *Mimusops zeyheri* seed, which is indigenous to southern Africa, *Q. robur* seed had a low concentration of the two minerals (Chivandi *et al*., 2011) showing that it is a poor source of both calcium and phosphorus. Despite *Q. robur* seed's low calcium and phosphorus, its ash content compares favourably with that of the *M. zeyheri* seed (Chivandi *et al*., 2011). The seed of *Adansonia digitata*, another indigenous tree, is reported to contain 41% of calcium (Osman 2004), which is much higher compared to that of *Q. robur* seed.

The phytate-phosphate in the *Q. robur* seed at 0.07% is much lower compared to the range of 0.2 to 5.36% reported for several cereal and legume grains (Coulibaly *et al.*, 2011). Dietary phytic acid, the major storage form of phosphorus in seeds, accounts for over 80% of the seed phosphorus (Bohn *et al.*, 2008; Raboy, 2009). In the gastrointestinal tract dietary phytic acid strongly chelates divalent and trivalent metal ions (Ca, Fe, K, Mg, Mn and Zn) making them unavailable for absorption in humans and animals (Bohn *et al*., 2008). In addition to creating nutritional deficiencies of the minerals that are chelated (Brown & Solomons, 1991), the egestion of phosphorus-rich organic waste results in the pollution of water resources (Brinch-Pedersen *et al*., 2002; Raboy, 2009). Although *Q. robur* seed has a lower phosphorus content compared to other farmed grain species (Coulibaly *et al*., 2011); its lower phytate-phosphate content could mean better availability of the phosphorus should it be used in foods and feeds. Thus the use of *Q. robur* seed meal in foods and feeds as an energy source could help in reducing environmental pollution from phosphorus-laden waste. The fibre content (NDF: 24.7% an ADF: 3.61%) of *Q. robur* seed are all higher than the 10.8% and 2.8% NDF and ADF, respectively reported *for Zea mays* (FAO, 1992). There is a physiological limitation for humans and monogastric animals to digest high fibrous foods and feeds (Chivandi *et al*., 2011), thus the potential use of *Q. robur* seed meal as a dietary energy sources needs to be considered in light of its relatively higher fibre content. One way to mitigate the negative effects of the observed higher fibre content would be to complement a traditional cereal, such as maize, with *Q. robur* meal in dietary formulations.

Table 3. Fatty acid profile of *Quercus robur seed oil*.

Fatty acid	Percent (%)
Saturated fatty acid	
Lauric acid (C12:0)	0.02 ± 0.00
Myristic acid (C14:0)	0.29 ± 0.02
Pentadecanoic acid (C15:0)	0.01 ± 0.00
Palmitic acid (C16:0)	14.50 ± 0.44
Margaric acid (C17:0)	0.15 ± 0.01
Stearic acid (C18:0)	1.84 ± 0.00
Arachidic acid (C20:0)	0.34 ± 0.01
Behenic acid (C22:0)	0.88 ± 0.67
Tricosanoic acid (C23:0)	0.09 ± 0.07
TSFA	**18.18**
Monounsaturated fatty acid	
Tetradecenoic acid (C14:1)	0.04 ± 0.00
*Pentadecenoic acid (C15:1)	0.02
Palmitoleic acid (C16:1)	0.46 ± 0.02
Heptadecenoic acid (C17:1)	0.11 ± 0.00
*cis-Oleic acid (C18:1)	41.06 ± 0.10
trans-Oleic acid (C18:1)	0.18
Gadoleic acid (C20:1)	0.06 ± 0.01
Erucic acid (C22:1)	0.09 ± 0.07
Nervonic acid (24:1)	0.02 ± 0.02
TMUFA	**42.04**
Polyunsaturated fatty acid	
Linoleic acid (C18:2n6c)	28.71 ± 0.17
Linoleic acid (C18:2n6t)	0.03 ± 0.01
α-Linolenic acid (C18:3n3)	1.94 ± 0.01
γ-Linolenic acid (C18:3n6)	0.26 ± 0.06
Dihomo- γ-Linolenic acid (C20:3n6)	0.03 ± 0.03
*Eicosatrienoic acid (C20:3n3)	0.01
*Arachidonic acid (C20:4n6)	0.02
*Eicosapentaenoic acid (C20:5n3)	0.04
Docosahexaenoic acid (C22:6n3)	0.03 ± 0.02
TPUFA	**30.22**
Cis Fats	69.77
Trans fats	0.08
Omega 3	2.14 ± 0.28
Omega 6	29.07 ± 0.11
Omega 9	41.23 ± 0.08
PUFA:SFA	**1.7:1**

TSFA = total saturated fatty acids; TMUFA = total monounsaturated fatty acid; TPUFA = total polyunsaturated fatty acids, Data presented (where applicable) as mean±SD, n = 3. In terms of the notation used in the fatty acid profiling the letter C and the number immediately after it represent the number of carbon atoms in the fatty acid molecule; the second number after the colon represents the number of double bonds in the fatty acid; n3, n6, n9 indicate position of the first double bond in a given fatty acid molecule.*Only one value read from the three replicates

Fatty acid profile of *Quercus robur:* Maki *et al.*, (2015) reported that oleic acid made up 28.4% of the lipid content of maize grain. At 41.06% of the seed lipid content, the oleic acid content of *Q. robur* seed is much higher compared to that of whole maize grain. The oleic acid

content of *Quercus brantii*, is reported to range from about 53to 66% (Khadadoust *et al.,* 2013), thus making our reported oleic acid content to be within the range of other seeds from the genus *Quercus*. The consumption of oleic acid is associated with health beneficial effects that accrue due to its (oleic acid) ability to reduce blood lipids including cholesterol (Lopze-Huertas, 2010. The reduction of blood lipids reduces the risk of cardiovascular diseases (Lopez-Huertas, 2010). The *Q. robur* seed oil also contained the essential fatty acids linoleic acid (28.71%) and α-linolenic (1.94%). Our results show that the linoleic fatty acid content of *Q. robur* seed meal (28.71%) to be higher than the 2.45% linoleic fatty acid content of the edible pit of *Sclerocarya birrea* (Glew *et al.,* 2004). Importantly both the linoleic and the α-linolenic fatty acid content of the *Q. robur* seed oil from our study are higher than that from Quercus brantii seed oil (Khadadoust *et al.,* 2013). These EFAs are required for normal growth and development (Needleman *et al*., 1979; Simopolous, 1991) and for the maintenance of neuronal membrane integrity (Holman, 1986; Holman 1998). The presence of oleic acid and EFAs in *Q. robur* seed oil could be potentially exploited by using the seed as a dietary supplement especially in resource limited communities.

Conclusion

Due to the high energy content and a high concentration of oleic acid and the EFAs linoleic acids and α-linolenic acid, *Q. robur* seed could potentially be exploited as a healthful dietary energy supplement especially in resource limited communities of South Africa.

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