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Impact of plant age and harvest season on nutritional profiles of *Podocarpus usambarensis* oils: Insights from Kahuzi-Biega National Park

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ABSTRACT

Introduction

The rising consumption of edible vegetable oils underscores the importance of understanding their nutritional value, particularly in terms of fatty acid composition and vitamin E content. *Podocarpus usambarensis* is a plant that grows in Kahuzi-Biega National Park in the Democratic Republic of the Congo. It produces oils used by the indigenous population for cooking and medicinal purposes.

Purpose

This study examines the impact of plant age and harvest season on the fatty acid and tocopherol content of *Podocarpus usambarensis* oil, highlighting significant differences based on these variables and their implications for public health nutrition.

Methods

Three samples of approximately 500 g of oilseeds were collected from young and old plants in the dry season, and from young plants in both the dry and rainy seasons. After drying, crushing, and powdering the collected seeds, oil was extracted using Soxhlet's procedure. A drop of oil was methylated, and aliquots were injected into a Varian 5890 gas chromatograph for fatty acid and tocopherol analysis.

Results

The fatty acids found in the oil were monounsaturated, including oleic and palmitoleic acids; polyunsaturated, including eicosadienoic, eicosatetraenoic, linoelaidic, linoleic, and linolenic acids; and saturated, principally behenic, cerotic, palmitic, and stearic acids. Total saturated fatty acids varied between 10 and 13%. Young trees were significantly ($p = 0.043$) richer in monounsaturated fatty acids than polyunsaturated ones (53.14% vs. 31.46%), while in old trees, there was no significant difference between polyunsaturated and monounsaturated fatty acids (40.68% vs. 40.59%).

Conclusion

The plant is an essential source of healthier nutrients, i.e., oleic acid, linoleic acid, and tocopherols. Plant age and seasonal climate impact the yields and composition of these nutrients in *Podocarpus usambarensis*.

INTRODUCTION

In the field of public health, almost one-third of the world's population—mostly women and children—lack at least one vital nutrient, which can be obtained through oilseed consumption (Riediger et al., 2008). Essential fatty acids (EFAs) included in vegetable oil are crucial for maintaining good health and preventing disease. The consumption of edible vegetable oils has been steadily rising due to urbanization, increases in per capita income, and shifts towards obesogenic diets worldwide (Tian et al., 2023). Vitamin E, comprising tocopherols and tocotrienols, is exclusively obtained from the diet and is effective against numerous diseases, including cancer, aging, arthritis, and cataracts (Rizvi et al., 2014). The nutritional values of oil are mainly determined by its fatty acid composition and vitamin E content (Guo et al., 2022).

Black cumin seeds, for instance, are nutritionally rich and have applications in the food, pharmaceutical, and cosmetic industries, primarily due to their fatty acid composition dominated by linoleic acid and their high levels of tocopherol (Albakry et al., 2022). Interest in unconventional vegetable oils rich in compounds with high biological value has increased (Nogala-Kalucka et al., 2010; Daud et al., 2022; Beres et al., 2017). For example, the analysis of grape pomace oil revealed it to be rich in bioactive compounds such as fatty acids and tocopherols (Carmona-Jiménez et al., 2022). A high content of unsaturated fatty acids (UFAs) and lower PUFAs was found in all the analyzed samples, leading the authors to suggest that this oil can be considered a source of vitamin E, with beneficial health implications.

There are two families of essential fatty acids: ω-3 (n-3) and ω-6 (n-6). These were initially designated as vitamin F when they were discovered as essential nutrients, but it was later shown by Burr et al. (1930) that they should be classified with fatty substances rather than vitamins (Zamora, 2005). Essential fatty acids cannot be synthesized in the human body from other nutrients or by any natural process, and must therefore be obtained through diet alone.

In a previous study, oleic acid was identified as the dominant fatty acid in *P. usambarensis* seed oil (Kazadi et al., 2014). This acid is known as one of the healthier sources of fat in the diet (Ellis-Christensen, 2009; Schuchardt et al., 2024), with benefits including the reduction of total cholesterol, slowing the development of heart disease, and providing antioxidant effects (Pérez-Jiménez et al., 2002). Oleic and erucic acids have also been used in the treatment of adrenomyeloneuropathy, a cerebral disease (Aubourg et al., 1993; Goyal et al., 2024). Furthermore, oleic acid is a component of various products, such as soaps and cosmetics, where it serves as a great moisturizer (Ellis-Christensen, 2009). Dubois et al. (2007) noted that olive oil, where oleic acid is the main component, might have a slight and controversial positive effect on LDL cholesterol, but other molecules might also play a role in these health benefits.

Higher intakes of very long-chain fatty acid (VLCFA) omega-3 have been associated with better health outcomes across many systems. Clinical studies have shown that higher blood levels of some VLCFA omega-3 are linked with reduced mortality, improved cardiovascular health, reduced preterm births, and better regulation of the normal immune response. Long-chain polyunsaturated fatty acid intake has also been associated with eye health, brain development, memory function in older adults, and mental health (Schuchardt et al., 2024). Tocopherols and tocotrienols, natural forms of vitamin E, act as powerful antioxidants alongside vitamin C and glutathione, with anti-inflammatory, antimutagenic, anticarcinogenic, and antitumor properties that contribute to their chemopreventive capacity for various pathologies (Surth, 2002). Through this antioxidant activity, they protect body tissues against free radical damage (Matthaus & Özcan, 2014).

To prevent the oxidation of PUFAs, there are physiological requirements for vitamin E of 0.6 to 1 mg/g of PUFAs (Dupin & Debry, 1982), making the intake of foods containing antioxidants necessary (Devron et al., 1995). Additionally, higher intakes of vitamin E are recommended to combat the peroxidation phenomena that occur in atherothrombosis (Stephens et al., 1996). Plant oils are the major dietary source of these provitamins E (Hammond, 2003). Interestingly, heating during food preparation does not deteriorate the antioxidant capacity of tocopherol; on the contrary, it increases this activity (Tangkanakul et al., 2011). This has led to a growing interest in alternative sources of tocopherols (Dauqan, 2011).

Due to seasonal variation and soil conditions, oils from different botanical types of a given plant can differ considerably in their composition. The yield and composition of vegetable oils often depend on the variety and climatic conditions, which can influence both the quantity and quality of the fatty acids produced (Calvaruso et al., 2006; Fleiss et al., 2022; Woittiez et al., 2017). For instance, Zineddine et al. (2006) reported that the total lipids and fatty acid composition of *Lygeum spartum* L. leaves exhibit a seasonal quantitative variation, with low lipid levels consisting primarily of saturated fatty acids (SFAs) during the period of vegetative dormancy, and higher levels of lipids with a prevalence of unsaturated fatty acids (MUFA, PUFA) in spring.

When investigating the effect of growing region and maturity stages on plant oil, Ziyad et al. (2021) observed the highest tocopherol isomer contents at the immature stage of *Pistacia atlantica*. In the case of palm oil, rainfall affects crop production throughout the year. Depending on the rainfall regime, mill activity might need to slow down, while a high level of solar radiation is vital for growth and fruit bunch production (Ochs, 1977). A fresh fruit bunch weighs, on average, 20–30 kg, depending on the age of the tree. In the first 3–4 years, the production of young palms is often small, with full production starting from the sixth year onwards and beginning to decline when the trees are 20–22 years old (Verheye, 2010).

Modification of fatty acid composition has been the primary goal of agricultural breeding programs. Understanding the combined effects of climatic fluctuations and plant age on oil composition would be useful for designing management practices aimed at obtaining a specific oil quality and improving predictions of crop models (Kasampalis et al., 2018; Andrew et al., 2018). In the Democratic Republic of the Congo, many plant species from the equatorial rain forest are worthy of domestication for oil production. *Podocarpus usambarensis* Pilger (Podocarpaceae), known as Omufa in Mashi, Podo in English, and Musenene in Luganda, is a very large evergreen tree that can reach up to 60 m in height, with solitary fruits containing a thin pulp surrounding a seed (Katende et al., 1995).

In samples collected at Kahuzi-Biega National Park and surrounding areas, rare fatty acids were identified, including cis-Δ5-unsaturated polymethylene-intermittent fatty acids (Δ5-AGIPIs), 5,11,14-20:3 (sciadonic acid), and 5,11,14,17-20:4 (juniperonic acid) (Kazadi et al., 2011). These acids are confirmed to be present because they are typically found in gymnosperms like this *Podocarpaceae* at a rate of about 5% of the total fatty acids (Mogrand et al., 2001; Wolff & Christie, 2002).

The Kahuzi-Biega National Park (KBNP) is located in eastern DRC at the crossroads of the Guineo-Congolese and Afromontane centers of endemism. It is a World Heritage site and one of the eight national parks of the Democratic Republic of the Congo, covering over 6000 km² between 1° 36'-2° 37' South latitude and 27° 33'-28° 46' East longitude (Yamagiwa et al., 2008). The present study aimed to compare the content of fatty acids and vitamin-E-active compounds (tocopherols) between young and old *Podocarpus usambarensis* trees, specifically analyzing seasonal variations. This comparison was conducted to determine how the age of the plant and the time of harvest influence the nutritional composition of the oil extracted from its seeds. Given the potential health benefits of these compounds, understanding these variations could guide better agricultural practices, optimizing the harvesting times for maximum nutritional yield and contributing valuable information to the field of public health nutrition.

METHODS

Study Area

The study was conducted in the Kahuzi-Biega National Park (KBNP) and surrounding areas, located in eastern Democratic Republic of the Congo. The park lies at the intersection of the Guineo-Congolese and Afromontane centers of endemism, providing a unique ecosystem with a wide variety of plant species. The climate in the region is tropical, with distinct wet and dry seasons, which significantly influence the growth and development of plant species, including *Podocarpus usambarensis*.

Plant Materials and Categorization of Plants as Old or Young

To identify plant type (old or young trees), the diameter at breast height (DBH) was measured at 1.3 m above the ground or immediately above the buttresses if these extended beyond 1.3 m. The DBH of the *Podocarpus usambarensis* trees measured 1.040 ± 0.034 m for old trees and 0.518 ± 0.065 m for young trees.

Categorization of Seasons as Dry and Rainy

The climate in the region where the target plants grow presents a short dry season (June–August) and a distinct long rainy season from September to May. A mean annual rainfall of 1,619 mm/year has been recorded, while the monthly mean temperature ranges from 18 to 20.5 °C (Yamagiwa et al., 2005).

Collection of Oilseeds

Plant oilseeds were collected in 2012 during both rainy and dry seasons from both old and young plants growing in the locality of Kabushwa, in the vicinity of eastern Kahuzi-Biega National Park (KBNP). Three oilseed samples from each plant were collected and taken to the Phytochemistry Laboratory of the "Centre de Recherche en Sciences Naturelles de Lwiro" (CRSN), South-Kivu, DR Congo, where they were dried for one week before being dried at 105°C in an oven (model Boekel from Arthur H. Thomas Co., Philadelphia, USA). At least 500 g of seeds were collected for each assay. Three samples of about 500 g of oilseeds were collected from young and old plants in the dry season, as well as from young plants in the dry and rainy seasons. After drying, crushing, and powdering the collected seeds, oil was extracted using Soxhlet's procedure. Seed samples were taken to the Phytochemistry Laboratory of the Centre de Recherche en Sciences Naturelles de Lwiro, where they were dried for one week before being dried at 105°C in an oven until a constant weight was achieved. After drying, the seeds were shelled by hand to remove the kernels, which were crushed using a coffee mill (model Corona 01 Landers & CIA. SA) to produce fine seed flour, as described elsewhere (Ghazi et al., 2013).

Extraction of Oil from Plant Oilseeds

Oil from the flour was extracted using Soxhlet's procedure (Barthet et al., 2002) by repeated washing with petroleum ether (boiling point 40 to 60°C). After 8 hours, the Soxhlet extraction flask containing the oil and solvent mixture was removed from the Soxhlet apparatus. The oil dissolved in petroleum ether was filtered using filter paper (Whatman No. 1), and the solvent evaporated under vacuum in a rotary evaporator (model Eyala, Tokyo Rikakikai Co. Ltd.). The remaining solvent traces were removed by heating the flask containing oil in a water bath at 90°C. The oil obtained was thereafter stored in hermetically closed bottles and kept in a refrigerator at 4°C until further analyses.

Analysis of Fatty Acids by GLC

The procedure was described elsewhere (Kazadi et al., 2011). Briefly, the temperature program was increased from 155°C to 220°C (1.5°C/min) with a 10-minute isotherm; injector and detector temperatures were set at 250°C; carrier gas was hydrogen at 36 cm/s; split ratio was 1:50; gas detector manual injection volume was less than 1 µL; and the carrier gas flow rates were 30 mL/min hydrogen, 300 mL/min air, and 30 mL/min nitrogen. The integration software computed the peak areas, and percentages of fatty acid methyl esters (FAME) were obtained as weight percents by direct internal normalization.

Following the ISO standard ISO 5509:2000 (DGF, 2014), a mixture of one oil drop dissolved in 1 mL of n-heptane and 50 µg of sodium methylate was introduced in a closed tube, agitated for 1 minute at room temperature, diluted with 100 µL of water, and centrifuged at 4500 g for 10 minutes. After removing the lower aqueous phase, 50 µL of HCl (1 mol with methyl orange) was added, and the mixture was quickly stirred before the lower aqueous phase was discarded. After centrifuging at 4500 g for 10 minutes, the top n-heptane phase was transferred to a vial and injected into a Varian 5890 gas chromatograph equipped with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 µm). Approximately 20 mg of sodium hydrogen sulfate (monohydrate, extra pure; Merck, Darmstadt, Germany) was added.

Determination of Vitamin-E-Active Compounds

For the determination of tocopherols and tocotrienols, the method DGF F-II 4a (00) (Codex, 2013) was used. Here, 250 mg of oil was dissolved in 25 ml of n-heptane and used for analysis in HPLC. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump (Merck-Hitachi, Darmstadt, Germany), a Merck-Hitachi F-1000 fluorescence spectrophotometer (Darmstadt, Germany; detector wavelengths for excitation 295 nm, for emission 330 nm), and a ChemStation integration system (Agilent Technologies Deutschland GmbH, Böblingen, Germany). Samples of 20 µL were injected by a Merck 655-A40 autosampler (Merck-Hitachi, Darmstadt, Germany) onto a Diol phase HPLC column 25 cm × 4.6 mm ID (Merck, Darmstadt, Germany) with a flow rate of 1.3 ml/min. The mobile phase used was 99 ml n-heptane + 1 ml tert-butyl methyl ether.

Data Analysis

A correlation matrix and tests of acids and tocopherols were carried out using GenStat release 7.1 (2003) and StartView software. All data were expressed as mean and standard deviation (mean ± SD). Statistical significance was established using a one-way analysis of variance (ANOVA) with the general linear means procedure. Significance was inferred when $p < 0.05$.

RESULTS

Figure 1 depicts the variations in fatty acids (FAs) with the age of the plant and the season of sample collection. The total monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) content are significantly higher ($p = 0.043$) in young plants than in old ones. Polyunsaturated fatty acids (PUFA) are higher in old plants compared to young plants. Total MUFA is more elevated in the dry season than in the rainy season, while PUFA and SFA are not significantly different between the two seasons.

Figure 1:
Variations of FAs with plant age and season of collection for *P. usambarensis*

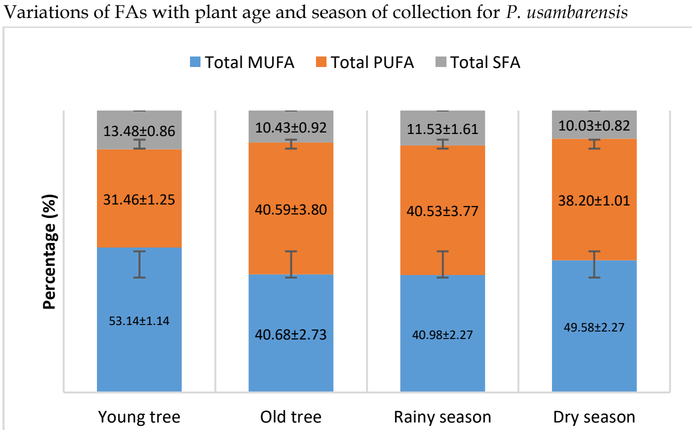

Table 1 shows the fatty acids detected in the oils of *P. usambarensis* species, which are subdivided into MUFA (eicosenoic acid, oleic acid, palmitoleic acid, vaccenic acid), PUFA (eicosenoic acid (11-), eicosadienoic acid (5,11-), eicosadienoic acid, eicosatetraenoic acid, eicosatetraenoic acid (5,11,14,17-), eicosatrienoic acid (5,11,14-), linoelaidic acid, linoleic acid, linolenic acid), and SFA (lignoceric acid, arachidic acid, behenic acid, cerotic acid, palmitic acid, stearic acid).

The majority of fatty acids were oleic acid (38.2–49.3%), linoleic acid (21.5–25.5%), eicosatetraenoic acid (5,11,14,17) (6.9–8.07%), and stearic acid (2.9–6.16%). By category, there were 10.03–13.48% SFA, 40.68–53.14% MUFA, and 31.46–40.59% PUFA. The production of total MUFA was higher in young trees (53.14±1.14%) and during the dry season (49.58±2.27%) compared to old trees (40.68±2.73%) and the rainy season (40.98±2.27%).

The production of PUFA, however, was higher in old trees (40.59±3.80%) and during the rainy season (40.53±3.77%) compared to young trees (31.46±1.25%). The concentration of total SFA was higher in young trees (13.48±0.86%) and during the rainy season (11.53±1.61%), compared to old trees (10.43±0.92%) and the dry season (10.03±0.82%), respectively.

Table 1:
Fatty acid content (percentage) in *P. usambarensis* oilseeds by age and season

Fatty acid	Young tree	Old tree	Rainy S.	Dry S.	P
Eicosenoic acid	0.89±0.51	0	0.43±0.50	0.42±0.18	Young, Rainy
Oleic acid	49.3±0.63	38.20±2.72	38.20±2.72	43.30±0.11	Young, Dry
Palmitoleic acid	-	-	-	3.12±1.80	-, Dry
Vaccenic acid	0.18±0.00	0.16±0.01	0.16±0.01	0.18±0.01	Young, Dry
Eicosénoic acid (11-)	2.77±0.07	2.32±0.16	2.32±0.16	2.56±0.07	Young, Dry
Total MUFA	**53.14±1.14**	**40.68±2.73**	**40.98±2.27**	**49.58±2.27**	**Young, Dry**
Eicosadiénoic acid (5,11-)	0.07±0.04	0.06±0.03	-	-	Young, -
Eicosadiénoic acid	1.23±1.54	4.30±0.50	4.30±0.50	4.21±0.18	Old, Rainy
Eicosatetraénoic acid	0.19±0.01	0.14±0.08	0.14±0.08	0.12±0.11	Young, Rainy
Eicosatetraénoic acid	6.93±0.08	8.070±0.68	8.07±0.68	8.02±0.24	Old, Rainy
Eicosatrinoic acid	0.39±0.24	1.95±1.07	1.95±1.07	1.02±0.58	Old, Rainy
Linoélaidicic acid	0.16±0.09	0.30±0.17	0.30±0.17	0.30±0.08	Old, Rainy
Linoleic acid	21.5±0.48	24.40±2.70	24.40±2.70	25.50±0.88	Old, Dry
Linolénic acid	0.99±0.07	1.37±0.17	1.37±0.17	1.59±0.14	Old, Dry
Total PUFA	**31.46±1.25**	**40.59±3.80**	**40.53±3.77**	**38.20±1.01**	**Old, Rainy**
Lignoceric acid	0.10±0.06	0.14±0.08	0.14±0.08	0.19±0.11	Old, Dry
Arachidic acid	0.77±0.08	0.38±0.02	0.38±0.02	0.40±0.02	Young, Dry
Behenic acid	2.17±0.11	3.63±0.36	3.63±0.36	3.55±0.29	Young, Rainy
Cerotic acid	1.95±1.07	1.02±0.58	2.12±1.99	-	Young, Rainy
Palmitic acid	2.31±0.09	2.31±0.28	2.31±0.28	2.57±0.12	Young, Dry
Stearic acid	6.18±0.88	2.95±0.39	2.95±0.39	3.32±0.18	Young, Dry
Total SFA	**13.48±0.86**	**10.43±0.92**	**11.53±1.61**	**10.03±0.82**	**Young, Rainy**
Total VLCFA	**14.97±1.89**	**22.22±1.27**	**22.22±1.27**	**19.95±0.75**	**Young, Rainy**

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As shown in **Table 2**, the total vitamin E active components varied between 2.54% and 5.87%.

Table 2:
Content of tocopherols (percentage) in *P. usambarensis* by age and season

Vitamin-E components	Young tree	Old tree	Rainy season	Dry season	P
	%	%	%	%	
α-Tocopherol	0.17±0.06	0.13±0.12	0.13±0.12	0	Young,Rainy
α-Tocotrienol	0.13±0.06	0.17±0.06	0.17±0.06	0.07±0.06	Old, Rainy
β-Tocopherol	3.17±0.31	2.97±2.05	2.97±2.05	1.73±1.46	Young,Rainy
γ-Tocopherol	1.17±0.25	0.63±0.45	0.63±0.45	0.03±0.06	Young,Rainy
Plastochromanol-8	0.07±0.06	0.07±0.06	0.07±0.06	0.07±0.06	Equality
γ-Tocotrienol	1.07±0.06	0.97±0.67	0.97±0.67	0.57±0.51	Young,Rainy
δ-Tocopherol	0.03±0.06	0	0.03±0.06	0.07±0.06	Young,Dry
δ-Tocotrienol	0.03±0.06	0.03±0.06	-	-	Equality -
Total	5.87±0.32	4.97±3.32	4.97±3.32	2.54±2.32	Young,-

DISCUSSION

The study aimed to determine whether the lipid composition of *Podocarpus usambarensis* oil varies with the plant's age and the season of harvest. The findings showed that the fatty acids (FAs) detected in the oil were monounsaturated fatty acids (MUFA), including eicosenoic acid, oleic acid, palmitoleic acid, and vaccenic acid; polyunsaturated fatty acids (PUFA), including eicosenoic acid (11-), eicosadienoic acid (5,11-), eicosadienoic acid, eicosatetraenoic acid, eicosatetraenoic acid (5,11,14,17-), eicosatrienoic acid (5,11,14-), linoelaidic acid, linoleic acid, and linolenic acid; and saturated fatty acids (SFA), including legnoceric acid, arachidic acid, behenic acid, cerotic acid, palmitic acid, and stearic acid. The production of total MUFA was significantly higher in young trees (53.14 ± 1.14%) and during the dry season (49.58 ± 2.27%) compared to old trees (40.68 ± 2.73%) and the rainy season (40.98 ± 2.27%). Conversely, the production of PUFA was higher in old trees (40.59 ± 3.80%) and during the rainy season compared to young trees (31.46 ± 1.25%). The concentration of total SFA was higher in young trees (13.48 ± 0.86%) and during the rainy season (11.53 ± 1.61%) compared to old trees (10.43 ± 0.92%) and the dry season (10.03 ± 0.82%).

For individual fatty acids, *P. usambarensis* oil was predominantly composed of oleic acid (38%–49%), linoleic acid (21%–25%), 5,11,14,17-eicosatrienoic acid (6.9%–8.1%), behenic acid (2.2%–3.6%), palmitic acid (2.3%–2.6%), and stearic acid (2.9%–6.2%). As indicated in Table 1, oleic acid, which is dominant in this oil and is known to be one of the healthier fatty acids (Ellis-Christensen, 2009; Pérez-Jiménez et al., 2002; Schuchardt et al., 2024), showed favourable conditions for young plants and dry season harvest. Very long-chain fatty acids (VLCFA) such as 11-eicosenoic acid also exhibited favourable conditions in young plants and dry season harvest, while 5,11,14,17-eicosatrienoic acid and linoleic acid were favoured in old plants and rainy season harvest. Additionally, behenic acid, palmitic acid, and stearic acid showed favourable conditions for young plants during the dry season of harvest. Similarly, *Lygeum spartum* lipids are primarily composed of SFA during dormancy and higher levels of MUFA and PUFA during spring (Zineddine et al., 2006). These findings align with our results, where higher levels of MUFA and PUFA were observed during the dry season and in young trees compared to old ones.

In terms of health applications, the use of *P. usambarensis* oil warrants special attention due to its content of monounsaturated fatty acids, particularly oleic acid. Oleic acid, an omega-9 fatty acid, is considered one of the healthier fats in the diet (Ellis-Christensen, 2009). It is the most widely consumed fatty acid and is beneficial for health as it lowers total cholesterol levels, prevents the development of cardiovascular diseases, and serves as a precursor for antioxidant production (Pérez-Jiménez et al., 2002). Oleic acid has a hypotensive effect, especially when derived from olive oil, and may hinder the progression of adrenoleukodystrophy (ALD), a fatal disease affecting the brain and adrenal glands (Rizzo et al., 1989). Moreover, the value of *P. usambarensis* oil lies in the presence of very long-chain fatty acids (VLCFA), which are more commonly found in fish oil (Gurr, 1999; Wijendran & Hayes, 2004).

Regarding tocopherols and tocotrienols, which are natural forms of vitamin E (Aluyor et al., 2009) with chemopreventive properties against various pathologies (Surh, 2002), most food plants contain low to moderate levels of vitamin-E-active compounds (VEAC). These compounds are typically undetectable in palm oils, palm oleins, or other commercial oils (Gao et al., 2023). However, in this study, the total amount of VEAC in *P. usambarensis* seed oil was significantly higher in young plants (5.87 ± 0.32 mg/100 g) than in old plants (4.93 ± 3.32 mg/100 g). This observation aligns with findings in *Pistacia atlantica*, where the highest tocopherol isomer contents were reported in immature stages compared to mature stages (Ziyad et al., 2021). The γ-tocopherols, which have a higher antioxidant effect (Fernández-Ventoso et al., 2022),

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were also found in significantly higher amounts in young trees (1.17 ± 0.25 mg/100 g) compared to old trees (0.63 ± 0.45 mg/100 g). However, for α-tocopherol and β-tocopherol, there was no significant difference between old and young trees. Plastochromanol-8, which is a major tocopherol component in olive oil (Matthäus & Özcan, 2011), was present only in trace amounts, with the same content (0.07 ± 0.06 mg/100 g) in both young and old trees. Kazadi et al. (2011) identified in *P. usambarensis* seed oil cis-D5-unsaturated polymethylene-interrupted fatty acids (D5-UPIFA), including 5,11,14-20:3 (sciadonic acid) and 5,11,14,17-20:4 (juniperonic acid). The presence of these fatty acids was anticipated, as these and other polymethylene-interrupted fatty acids are commonly found in all gymnosperms, typically constituting around 5% of the total fatty acids, with sciadonic acid being the most abundant (Mogrand et al., 2001; Wolff & Christie, 2002). To date, 5-UPIFAs have been identified in 6 out of the approximately 100 *Podocarpus* species (Mogrand et al., 2001; Wolff et al., 1999; Bagci & Sahin, 2004). The seed oils of *P. usambarensis* appear to hold dietary and medicinal value due to their profile of essential fatty acids, particularly omega-3 and omega-6 (Kazadi et al., 2014).

CONCLUSION

Podocarpus usambarensis old trees produce more polyunsaturated fatty acids during the rainy season compared to young trees, while young trees in the dry season produce higher levels of monounsaturated fatty acids. This plant is a significant source of healthy fatty acids, such as oleic and linoleic acids, and the plant's age and seasonal climate influence the yields and composition of these nutrients. Additionally, *P. usambarensis* seed oil contains higher amounts of tocopherols, which are effective against various diseases, including cancer, aging, arthritis, and cataracts, in young plants compared to old ones.

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