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Traditional seed dehulling practices promote oxidation of *Ricinodendron heudelotii* kernel oil rich in conjugated linolenic acids

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ABSTRACT

Conjugated linolenic acids (CLnAs) are attracting growing scientific interest due to their promising anticancer properties. However, these conjugated fatty acids are scarce, as they are primarily found in the oils of a limited number of plant species. Notably, the oil extracted from *Ricinodendron heudelotii* (ricinodendron) kernels contains high proportions of α-eleostearic acid. Despite its widespread consumption across West and Central Africa, the precise composition and oxidative sensitivity of this oil remain largely unexplored. Yet, its high CLnA content, combined with the pro-oxidant nature of the traditional seed dehulling process, raise concerns about the oxidative quality of the kernels and oils sold in Africa. In the present study, we investigated the oxidative impacts of different dehulling methods on ricinodendron oil. Ricinodendron kernels dehulled using traditional methods, whether purchased as such or obtained by reproducing the traditional process under controlled laboratory conditions, exhibited elevated levels of oxidation, while directly dehulled kernels were only marginally oxidised. In addition, we analysed the composition of ricinodendron oils from several communes of Benin. Our results highlight ricinodendron kernel oil as a promising source of CLnAs for humans but underscore the need to adapt traditional dehulling practices to prevent the formation of potentially harmful oxidation products.

1. Introduction

Ricinodendron heudelotii (Ricinodendron) is a wild, non-domesticated tree species widespread across sub-Saharan Africa (Vivien and Faure, 1985). It is known as "akpi" in Ivory Coast (Nikiema et al., 2024) and "njanssang" in Cameroon (Ndumbe et al., 2019). This plant is found on the edges of tropical forests or in wooded savannahs (Vivien and Faure, 1996) and is used in intercropping systems as a shade plant, notably with cocoa trees, or in agroforestry in certain cases (N'Zi et al., 2023; Onefeli et al., 2019). Ricinodendron produces plump fruits that contain two to three seeds, composed of a ~1 cm kernel protected by a sclerotic envelope (Tchoundjeu et al., 2006). Seed dehulling is an arduous task, yielding kernels that are consumed as sauce thickeners and flavour enhancers in traditional African dishes (Adome et al., 2022; Tchiégang et al., 1997) and represent an economically important non-timber forest product for the subsistence of rural communities (Cosyns et al., 2011, 2014; Hounsou-Dindin et al., 2022; Ndumbe et al., 2019). They consist of approximately 50% oil (Tchiégang et al., 1997), that is extracted by

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mechanical pressing or solvent extraction (Dandjouma et al., 2008; Tchiégang et al., 2003, 2005) and is used in traditional medicine as a skin ointment (Hounsou-Dindin et al., 2022). Ricinodendron oil is of particular biological interest, as it contains elevated amounts of conjugated linolenic acids (CLnAs), primarily α-eleostearic acid (α-ESA – C18:3 *c9t11t13*), which can account for up to 60% of the oil's total fatty acids (Nikiema et al., 2019).

The term CLnA refers to a group of isomers of α-linolenic acid (ALA – C18:3 *c9c12c15*), in which at least two of the three double bonds are conjugated (Dhar Dubey et al., 2019). CLnAs bearing two conjugated double bonds are mostly produced through the isomerisation of ALA by rumen bacteria (Destaillats et al., 2005; Salsinha et al., 2018) and CLnAs containing three conjugated double bonds are found in high concentrations in peculiar plant seed oils. Their conjugated double bonds are found in *cis* and *trans* configurations, either in positions 9–11–13 or 8–10–12 of the fatty acid carbon chain, giving rise to seven different plant-derived CLnA isomers. Among them, the most studied isomers are α-ESA, notably found in tung seed oil *(Aleurites fordii)* (Li et al., 2012) and punicic acid (PunA - C18:3 *c9t11c13*), found in pomegranate seed oil (*Punica granatum*) (Melo et al., 2016). In recent years, CLnAs have attracted increasing scientific interest due to their potential health benefits (Du et al., 2024). In particular, their strong anti-tumour properties have been studied for more than 25 years (Dhar Dubey et al., 2019; Igarashi and Miyazawa, 2000; Suzuki et al., 2001; Tsuzuki et al., 2004). More recently, these effects have been linked to ferroptosis (Beatty et al., 2021; Cuvelier et al., 2025; Vermonden et al., 2021, 2024), a regulated form of cell death driven by membrane lipid peroxidation (Dixon and Olzmann, 2024; Hirschhorn and Stockwell, 2019).

Lipid oxidation has been extensively studied for over 60 years (Schultz, 1962). Not only responsible for unpleasant aromas in fat-containing foods, it also contributes to nutritional alteration and formation of harmful compounds (Gharby et al., 2025). Lipid oxidation is typically divided in two major phases: primary and secondary oxidation. Primary oxidation encompasses the processes whereby lipids are converted into hydroperoxides in a chain reaction involving molecular oxygen. This process, mainly occurring as autoxidation in vegetable oils, is typically divided in three steps: initiation, propagation and termination. In vegetable oils, initiation can be promoted by pro-oxidative factors such as heat, light or metal ions (Gharby et al., 2025; Machado et al., 2023). Although hydroperoxides are unstable, they can accumulate to some extent in oxidised vegetable oils and are considered as the main products of primary oxidation. In addition to autoxidation, photo-oxidation mediated by singlet oxygen may also contribute to hydroperoxide formation in vegetable oils exposed to light and containing photosensitizers such as chlorophylls (Gharby et al., 2025; Suhag et al., 2024). Secondary oxidation encompasses the degradation of lipid hydroperoxides into a wide range of lower-molecular-weight compounds, including aldehydes, ketones and alcohols (Frankel, 1984; Grebenteuch et al., 2021; Yin et al., 2011). Some of those molecules (*i.e.* α,β-unsaturated aldehydes), found in oxidized vegetable oils (Douny et al., 2015), are of particular biological concern because of their high electrophilicity. They can form covalent adducts with nucleophilic sites on DNA and proteins, thereby contributing to oxidative stress, inflammation, and potentially carcinogenic effects (Nakamura and Nakamura, 2020; Ng et al., 2014).

Oxidation pathways of conjugated fatty acids, and particularly of CLnAs, are still poorly understood. However, their high oxidative sensitivity has been documented by different research groups (Tsuzuki et al., 2004; Yamamoto et al., 2014; Yang et al., 2009). Do and co-workers measured the equilibrium rate constant of propagation — *i.e.* the autoxidation rate-limiting step — for several lipid species, and estimated that CLnAs were more than 8 times more susceptible to oxidation than their non-conjugated counterpart (Do et al., 2021). It has also been demonstrated that CLnAs tend to form so-called peroxide polymers upon autoxidation (Do et al., 2023; Do and Xu, 2023). "Unzipping" of these polymers has been proposed as a source of peculiar aldehydes production, namely α,β,γ,δ-unsaturated aldehydes or conjugated dienals, which are of biological relevance due to their high electrophilicity and potential toxicity (Do et al., 2023). According to this recent literature, CLnA-rich products such as ricinodendron kernels and oil should be handled, processed and stored with care. In that respect, traditional practices used in Africa for ricinodendron seed dehulling raise concern regarding their potential impact on lipid oxidation.

Ricinodendron seed collection and dehulling is a labour-intensive and time-consuming process, mostly carried out by women in rural communities (Coulibaly et al., 2018). After fruit collection and natural pulp degradation, the seeds are washed in water and boiled over a wood fire. This boiling step lasts from 8 h to multiple days, during which ricinodendron seeds crack open, therefore greatly facilitating their subsequent dehulling. Once shelled with the help of a sharp tool, ricinodendron kernels are sun-dried for several days. This drying step serves a double objective: removing excess moisture resulting from the boiling process and developing the kernels' characteristic aromas and golden-brown colour. After drying, ricinodendron kernels are sold in local markets or used for oil extraction. While minor variations exist among rural communities and countries — particularly regarding the duration of fruit fermentation, seed boiling, or kernel sun-drying — the overall traditional dehulling process remains remarkably consistent across West and Central Africa (Coulibaly et al., 2018). Despite its extensive use, this process is quite inefficient and physically demanding, and no appropriate technology has so far been adopted to alleviate its drudgery (Charlie et al., 2013). More importantly, prolonged exposure to pro-oxidative factors such as heat, light and water during the traditional dehulling process may have detrimental effects on the highly oxidation-prone ricinodendron kernel lipids (Nikiema et al., 2019).

Despite the suspected susceptibility of CLnA-rich ricinodendron oil to oxidation, there is currently a lack of experimental data linking traditional post-harvest dehulling practices to measurable changes in the oil. In addition, available information on the chemical composition of ricinodendron oil remains scarce and fragmented, particularly at the national scale in Benin. The primary objective of the present study was therefore to assess the impact of key steps of the traditional dehulling process on the oxidative status and composition of ricinodendron oil by reproducing these treatments under controlled laboratory conditions. In addition, the precise composition — in terms of fatty acids, tocopherols, tocotrienols and phytosterols — of ricinodendron oils obtained from seeds collected in different communes of Benin was characterised.

2. Materials and methods

2.1. Chemicals and reagents

All solvents and reagents used were either GC, HPLC or analytical grade. Organic solvents including *n*-hexane, diethyl ether, methanol, iso-octane, chloroform and glacial acetic acid were purchased from VWR Chemicals (Radnor, PA, USA). Potassium iodide, sodium thiosulfate, and other chemicals used for peroxide value determination were obtained from Sigma-Aldrich (St. Louis, MO, USA). *p*-anisidine was purchased from ThermoFisher Scientific (Waltham, MA, USA). Details on analytical standards used for fatty acid, tocopherol-tocotrienol and phytosterol chromatographic analyses are provided in the corresponding analytical sections.

2.2. Ricinodendron seeds and kernels

Ricinodendron seeds originating from different communes of Benin (Adja-Ouèrè, Bantè, Bassila (2 samplings), Kétou-Lama, Pobè) were kindly provided by the University of Abomey-Calavi (Abomey-Calavi, Benin) and the National University of Agriculture (Cotonou, Benin). The seeds were obtained from ripe ricinodendron fruits, after flesh removal.

Traditionally dehulled ricinodendron kernels were purchased on the Dantokpa market (Cotonou, Benin). No information was available

regarding their storage time and conditions, or their provenance. These processed kernels are hereinafter referred to as "market kernels".

All ricinodendron material (seeds and market kernels) was shipped to the LIBST, UCLouvain (Louvain-la-Neuve, Belgium) after collection, and preserved under vacuum at −20°C prior to analysis. Each ricinodendron seed batch was divided into three independent experimental replicates, which were processed separately throughout the experiments, resulting in three non-pooled oil samples after seed dehulling and oil extraction. Origin, processing methods and experimental roles of all ricinodendron material used in this work are presented on Table 1.

2.3. Ricinodendron seed dehulling and kernel oil extraction methods

Ricinodendron seeds were dehulled using two methods: by water boiling, upon reproduction of the traditional dehulling process under controlled laboratory conditions, or by direct dehulling, with a nutcracker (L'AR boutique, Pont-À-Mousson, France). Ricinodendron oil was extracted from the kernels using two methods: mechanical, with an oil press, and chemical, by Soxhlet extraction. These methods of seed dehulling and oil extraction were used distinctly depending on the respective experiment carried out. First, ricinodendron seeds from Bassila 1, available in greater quantities, were used for the reproduction of the traditional dehulling process. They were dehulled via water boiling (or direct dehulling for control samples) and oil extraction was performed by mechanical pressing. Second, ricinodendron seeds from Adja-Ouèrè, Bantè, Bassila 2, Kétou-Lama and Pobè, available in smaller quantities, were used for the characterisation of the geographical variability of ricinodendron oil across different communes of Benin. These seeds were dehulled via direct dehulling and oil extraction was performed by Soxhlet.

2.3.1. Reproduction of traditional dehulling process and mechanical oil extraction

The traditional ricinodendron seed dehulling process was reproduced under controlled laboratory conditions to evaluate its impacts on the composition and oxidative status of the oil. Seeds were boiled in water for 8 h on a hot plate, after which small cracks formed on the surface of the shells. Once dehulled, boiled kernels were dried in an incubator at 35°C under UV–visible-light, to mimic the traditional sun-drying step. A 36 W solar lamp (ReptiSun, San Luis Obispo, CA, USA) was used due to its similarity with natural sunlight in terms of spectrum and its high UV emission (10% UVB (280–320 nm), 30% UVA (320–400 nm) and 60% visible light (mostly 400–450 nm)). Kernels were recovered after 0, 3 or 10 days of incubation, with or without light exposure. As detailed above, ricinodendron seeds from Bassila 1 were used for this experiment. Non-boiled Bassila 1 seeds served as negative control. They were directly dehulled with a nutcracker (L'AR boutique, Pont-À-Mousson, France) without any prior treatment (see Section 2.3.2.). Conversely, traditionally processed market kernels served as positive control.

In this experiment, all ricinodendron oils were obtained by mechanical extraction using an oil press (Cgoldenwall, Beijing, China). To ensure satisfactory extraction yields, the press was preheated to 70°C. After pressing, ricinodendron oil samples were immediately analysed for oxidative status, then flushed with argon and stored at −20°C prior to further analyses.

2.3.2. Direct dehulling of ricinodendron seeds and chemical oil extraction

Ricinodendron seeds used for the characterisation of the geographical variability of ricinodendron oil across different communes of Benin (Adja-Ouèrè, Bantè, Bassila 2, Kétou-Lama and Pobè) were manually dehulled one by one without any prior treatment, using a nutcracker (L'AR boutique, Pont-À-Mousson, France). Chemical extraction was conducted with a Soxhlet apparatus, using diethyl ether as solvent, because of its low boiling point. Crushed ricinodendron kernels were placed in a cellulose thimble and inserted into the extractor. After 2 h of extraction in diethyl ether, the resulting solution was collected and evaporated using a rotavapor. The resulting ricinodendron oil samples were immediately analysed for oxidative status, then flushed with argon and stored at −20°C prior to further analyses.

2.4. Evaluation of the oxidative status of ricinodendron oils

2.4.1. Peroxide value

Primary oxidation was evaluated by the determination of the peroxide value (PV), according to the AOAC Official Method 965.33 (AOAC, 2000), with some modifications. First, an oil sample (2.5 g) was dissolved in 15 mL of a 3:2 (*v/v*) acetic acid/chloroform mixture. Subsequently, 0.25 mL of a saturated potassium iodide (KI) solution was added. The mixture was vigorously shaken and left in the dark for 5 min, to allow the reaction of the hydroperoxides present in the oil with the iodide ions (I^-) to form iodine (I_2). The reaction was stopped by the addition of 15 mL of distilled water. The I_2 was then titrated with either 0.1 *N* or 0.01 *N* $Na_2S_2O_3$, depending on the expected level of oxidation of the oil sample. Starch was used as a colour indicator, turning from dark blue to colourless upon I_2 consumption. Titration was stopped when no blue colour remained, and the PV was calculated using Eq. 1.

$$\mathbf{PV}\ (\mathbf{meqO_2/kg\ oil}) = \frac{(\mathbf{V} - \mathbf{V_b}) \times \boldsymbol{N} \times \mathbf{1000}}{\mathbf{m}} \quad (1)$$

V: volume of $Na_2S_2O_3$ for sample titration; V_b: volume of $Na_2S_2O_3$ for blank titration; *N*: $Na_2S_2O_3$ normality; m: mass of the oil sample

Table 1
Details regarding the ricinodendron material used.

Origin	Material	Dehulling method	Role	Oil extraction	Experiment
Dantokpa market	Traditionally processed kernels	Traditional dehulling (uncontrolled)	Positive control	Mechanical pressing	Reproduction of traditional dehulling
Bassila 1	Unprocessed seeds	Reproduction of traditional dehulling (controlled)	Experimental sample	Mechanical pressing	Reproduction of traditional dehulling
Bassila 1	Unprocessed seeds	Direct dehulling	Negative control	Mechanical pressing	Reproduction of traditional dehulling
Adja-Ouèrè	Unprocessed seeds	Direct dehulling	Experimental sample	Soxhlet extraction	Geographic compositional variability across Benin
Bantè	Unprocessed seeds	Direct dehulling	Experimental sample	Soxhlet extraction	Geographic compositional variability across Benin
Bassila 2	Unprocessed seeds	Direct dehulling	Experimental sample	Soxhlet extraction	Geographic compositional variability across Benin
Kétou	Unprocessed seeds	Direct dehulling	Experimental sample	Soxhlet extraction	Geographic compositional variability across Benin
Pobè	Unprocessed seeds	Direct dehulling	Experimental sample	Soxhlet extraction	Geographic compositional variability across Benin

For each experimental role, ricinodendron seeds and kernels were individualized in three independent experimental replicates. Extracted oil samples were not pooled.

2.4.2. p-anisidine value

Secondary oxidation was evaluated by the determination of the *p*-anisidine value (*p*-AV), according to the AOCS Official Method Cd 18–19 (AOCS, 1998). An oil sample (0.5 g) was dissolved in 25 mL of isooctane (2,2,4-trimethylpentane). A 5 mL aliquot of this solution was allowed to react with 1 mL of 0.25% (*w/v*) *p*-anisidine in acetic acid, in the dark, for 10 min. Absorbance was measured at 350 nm using a quartz cuvette, both before and after the addition of the *p*-anisidine reagent. Pure isooctane was used as a blank. The *p*-AV was calculated using Eq. 2.

$$\boldsymbol{p-AV} = \frac{\boldsymbol{25 \times (1.2 \times A_s - A_b)}}{\mathbf{m}} \quad (2)$$

A_s: absorbance of the oil sample, at 350 nm; A_b: absorbance of the blank at 350 nm; m: mass of the oil sample

2.4.3. Total oxidation

The Totox index was used as a global indicator of the total oxidation status of the oils under investigation. It consists of a combination of primary and secondary oxidation values. Totox was calculated using equation 3.

$$\mathbf{Totox} = \mathbf{2} \times \mathbf{PV} + \boldsymbol{p} - \mathbf{AV} \quad (3)$$

2.5. Quantification of fatty acids

Fatty acids were identified and quantified with a gas chromatograph coupled with a flame ionization detector (GC-FID). To allow their detection, fatty acids were derivatized into fatty acid methyl esters (FAMEs) via a two-step methylation, first under basic, then under acidic conditions. Oil samples were dissolved in 10 mL of 0.1 M potassium hydroxide in methanol (KOH/MeOH). After homogenization, samples were incubated at 70°C for 1 h and vigorously shaken every 20 min. Once cooled to room temperature, 4 mL of 1.2 M hydrochloric acid in methanol (HCl/MeOH) were added, and the mixture was incubated again at 70°C for 12 min. Following methylation, FAMEs were extracted overnight by the addition of 20 mL of hexane and 10 mL of Milli-Q water. Samples were analysed using a Trace 1310 GC (ThermoFisher Scientific, Waltham, MA, USA) equipped with an As Triplus autosampler (Thermo Electron, Milan, Italy) and a RT2560 capillary column of 100 m × 0.25 mm × 0.2 µm (Restek, Bellefonte, PA, USA). The oven temperature program was as follows: initial temperature of 80°C; increase at 25 °C/min up to 175°C; hold for 25 min; increase at 10 °C/min up to 200°C; hold for 20 min; increase at 10 °C/min up to 220°C; hold for 5 min; increase at 10 °C/min up to 235°C; hold for 15 min; decrease at 20 °C/min to initial temperature of 80°C. H_2 was used as the carrier gas. The FID was supplied with air (350 mL/min), H_2 (35 mL/min) and N_2 (40 mL/min) and set at 255°C. An internal standard, used to correct for experimental errors, was prepared from nonadecanoate (Larodan, Solna, Sweden) dissolved in chloroform at a concentration of 12.51 g/L. A volume of 0.5 mL of this internal standard was added in each oil sample, prior to methylation. Methyl-undecanoate (Larodan, Solna, Sweden) was used as an injection standard to correct for injection-related variability. Fatty acid identification and quantification were performed using an external standard mixture prepared from 45 individual FAME standards purchased from Larodan (Solna, Sweden). In addition, a CLnA standard was prepared from α-ESA FAME (Larodan, Solna, Sweden) and used for the quantification of CLnA peaks. CLnA concentrations are therefore expressed as α-ESA equivalents. Chromatograms were processed using Chromeleon 7.3 software (Thermo Fisher Scientific, Waltham, MA, USA). Data are presented as % of total fatty acids (*w/w*).

2.6. Quantification of tocopherols and tocotrienols

Tocopherols and tocotrienols were identified and quantified by high-performance liquid chromatography (HPLC), following the protocol of Nain et al. (2021), with some modifications. Oil samples were dissolved in hexane and filtered through 0.45 µm syringe filters into HPLC vials. Dissolved samples were injected on a HPLC (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an autosampler AS3000 (Oven temperature = 35°C, tray temperature = 15°C), a P1000 spectra system pump (Thermo separation products, San Jose, CA, USA), a Luna 5 µm normal phase column of 250 mm × 4.6 mm internal diameter (Phenomenex, Torrance, CA, USA) and an FP-2020 fluorescence detector (Jasco, Tokyo, Japan). The mobile phase consisted of 2% (*v/v*) tetrahydrofuran in *n*-heptane at a flow rate of 1.8 mL/min. Standard solutions containing increasing concentrations (1, 2, 5, 10, 50 and 100 ppm) of α-, γ-, δ-tocopherols (Sigma-Aldrich, St. Louis, MO, USA) and α-, γ-, δ-tocotrienols (Sanbio, Uben, The Netherlands) were used for the identification and quantification of the different forms of vitamin E. Chromatograms were processed using Chromquest 5.0 software (Thermo Fisher Scientific, Waltham, MA, USA). Data are presented as mg/kg of oil.

2.7. Quantification of phytosterols

Phytosterol identification and quantification was carried out following the method described by Vellemans et al. (2025). The unsaponifiable fraction of oil samples was obtained by a 2-hour incubation at 50°C, in KOH 2 M followed by extraction in hexane. Phytosterols were then derivatized to their trimethylsilyl (TMS) ether forms and analysed by a 6890 GC-FID (Agilent Technologies, Santa Clara, CA, USA) equipped with a 7683 Auto Sampler (Agilent Technologies, Santa Clara, CA, USA) and a Rxi-5Sil column of 30 m × 0.25 mm × 0.25 µm (Restek, Bellefonte, PA, USA) using N_2 as the carrier gas. The oven temperature program was as follows: initial temperature of 70°C; increase at 30 °C/min up to 290°C; hold for 22 min; increase at 30 °C/min up to 320°C; hold for 5 min. A plant sterol mixture (Cayman Chemicals, Ann Arbor, MI, USA) was used as an external standard for phytosterol identification and quantification, and betulin (Thermo Fisher Scientific, Waltham, MA, USA) was used as an internal standard. Chromatograms were processed using ChemStation B.04.01 software (Agilent Technologies, Santa Clara, CA, USA). Data are presented as mg/kg of oil.

2.8. Statistical analysis

All experiments were carried out on three independent experimental replicates. Results are expressed as mean ± standard deviation. All graphs and statistics were performed on GraphPad Prism 10 (Dotmatics, Boston, MA, USA). Data were analysed using Student's *t*-test or one-way ANOVA followed by Tukey's honestly significant difference (HSD) multiple comparison test, when assumptions of normality and homogeneity of variances were met. When these assumptions were not satisfied, non-parametric tests were applied, using the Kruskal–Wallis test followed by appropriate multiple comparisons. Differences between samples were considered statistically significant at $p \leq 0.05$.

3. Results and discussion

3.1. Oxidative status of ricinodendron oils from directly dehulled or traditionally processed kernels

Given the widespread consumption of ricinodendron kernels across West and Central Africa, it is paramount to evaluate their oxidative status. To this end, traditionally processed ricinodendron kernels purchased on the Dantokpa market (Cotonou, Benin) were compared with unprocessed kernels obtained by direct dehulling of ricinodendron seeds (Bassila 1). Ricinodendron oil was extracted from both kernel batches by mechanical pressing and assessed for primary and secondary oxidation by measuring the PV and the *p*-AV, respectively. Pictures of ricinodendron seeds and kernels, obtained by direct or traditional dehulling, are displayed on Fig. 1.

Oxidation results indicate markedly elevated levels of both primary

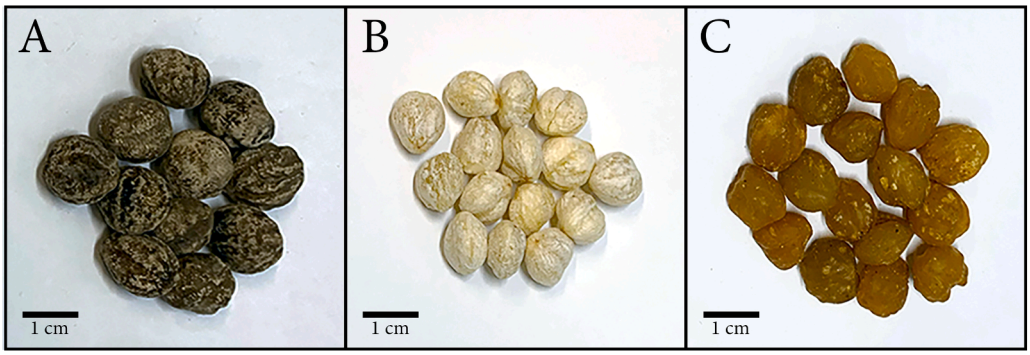

Fig. 1. Pictures of ricinodendron seeds (A) and kernels, either obtained by direct dehulling (B) or traditionally processed, *i.e.* boiled and sun-dried (C).

and secondary oxidation products in ricinodendron oil extracted from market kernels, with a PV of 27.89 meqO_2/kg and a *p*-AV of 87.13. In contrast, oil obtained from directly dehulled kernels could be considered virtually oxidation-free, with a PV of 0.69 meqO_2/kg and a *p*-AV of 2.84. Overall, the Totox index of the oil from directly dehulled kernels was more than 30 times lower than that of the traditionally processed oil from market kernels.

The PV is commonly used for the evaluation of hydroperoxide content (Gotoh and Wada, 2006), while the *p*-AV reflects the presence of aldehydic compounds, primarily 2,4-dienals and 2-alkenals (Tompkins and Perkins, 1999). The combined measurement of PV and *p*-AV is therefore recognized as a reliable indicator of the oxidative status of edible oils and is widely used in both industrial and academic settings (Irwin and Hedges, 2004). In addition to imparting undesirable flavours to oxidised foods, some oxidation products — especially α,β-unsaturated aldehydes — are known for their harmful effects on human health due to their high electrophilicity (Frankel, 1984; Grootveld et al., 2020; Jackson and Penumetcha, 2019; Leong, 2021; Ramana et al., 2013). The Codex Alimentarius sets maximum acceptable PVs at 15 meqO_2/kg for cold-pressed and virgin oils, and 10 meqO_2/kg for refined oils (Codex Alimentarius Commission, 1999). Although no threshold is established for *p*-AV, levels exceeding 20–30 are generally associated with substantial concentrations of secondary oxidation products. Considering these recommendations, the ricinodendron oil extracted from market kernels can be considered highly oxidised. Notably, the elevated *p*-AV indicates an important concentration of potentially deleterious aldehydes.

Additional results from our group, together with the limited literature available, indicate recurring high levels of oxidation in ricinodendron kernels from West and Central Africa. Reported PVs for ricinodendron oil typically range from 10 to 30 meqO_2/kg (Abaidoo-Ayin et al., 2017; Issaka et al., 2019; Kinge et al., 2019; Tchiégang et al., 2004). Data regarding secondary oxidation products are scarcer, but the few available reports show elevated *p*-AV or thiobarbituric acid (TBA) value (Issaka et al., 2019; Kinge et al., 2019). These high oxidation levels appear to be linked to the ricinodendron traditional seed dehulling process, although most studies do not specify whether the analysed oils were extracted from traditionally processed kernels. The pivotal role of the traditional dehulling is further supported by the low oxidation levels measured in the oil extracted from unprocessed kernels analysed in the present study, which displayed Totox values below 5. To note, this observation could also constitute evidence of the protective role of the shells that encapsulate ricinodendron kernels, sheltering the sensitive oil from pro-oxidative factors prior to seed dehulling.

3.2. Reproduction of the traditional dehulling process under controlled laboratory conditions

Despite its extensive use across West and Central Africa, the traditional dehulling process of ricinodendron seeds and its effects on the quality of the oil have been little studied. In 2018, Coulibaly et al. have investigated the impacts of the traditional treatment on the composition of defatted kernels, while Nikiema et al. (2019) reported changes in ricinodendron oil composition following seed boiling and kernel sun-drying. Additional studies have examined the effects of heat treatments (*e.g.* roasting, boiling, microwaving) on the oxidative status of already traditionally dehulled kernels (Ketaona et al., 2013; Kinge et al., 2019). However, to our knowledge, no research work has directly focused on the oxidation induced by the traditional process itself. To address this gap, we reproduced the key steps of this process under controlled laboratory conditions. Ricinodendron seeds (Bassila 1) were boiled and manually dehulled, after which kernels were incubated at 35°C either under a solar lamp emitting UV and visible light, or in the dark, for 0, 3 or 10 days. Oils were then extracted by mechanical pressing and analysed for oxidative status (PV, *p*-AV, Totox index) and for composition (fatty acids, tocopherols, tocotrienols). In comparison, oil samples from non-boiled, directly dehulled kernels from the same seeds (Bassila 1) were used as negative control, whereas oil extracted from market kernels was used as positive control for traditional processing. The oxidative status of the oils extracted after treatments is shown in Table 2, and changes in fatty acid and tocopherol-tocotrienol compositions are displayed in Fig. 2.

At day 0, oils from kernels obtained after seed boiling were not significantly more oxidised than oils from non-boiled, control kernels. After 3 days of incubation, only PV had significantly increased, both in light-exposed kernels and in kernels incubated in the dark ($p \leq 0.05$). After 10 days, oxidation progressed further, reaching a PV of 10.1 meqO_2/kg and a *p*-AV of 22.5 in light-exposed samples. Interestingly, the PV was higher in light-exposed kernels ($p \leq 0.05$), while the *p*-AV and Totox index were non-significantly different ($p > 0.05$) from kernels kept in the dark. Overall, these results indicate that ricinodendron kernel drying, as practiced in Central and West Africa and reproduced here under controlled laboratory conditions, has a substantial impact on the oxidative status of the oil contained in the kernels. Nevertheless, after 10 days of incubation, PV and *p*-AV remained lower than those measured in oil from market kernels, suggesting that our experimental setup may underestimate the extent of oxidation occurring under real-life conditions. The absence of a marked pro-oxidant effect of light exposure further suggests that either the intensity of the solar lamp was insufficient to fully mimic natural sunlight, or that ricinodendron oil contains relatively low levels of photosensitizers, thereby limiting the extent of photo-oxidative reactions. In addition, the higher oxidation levels quantified in the oil from market kernels may result from longer exposure times to heat, oxygen and sunlight during storage and sale. However, the lack of information regarding the history of market kernels prevents definitive conclusions.

Taken individually, tocopherols and tocotrienols all remained stable throughout the drying, with the exception of γ-tocotrienol, which dropped below the limit of detection in all treatments. No significant

Table 2
Impacts of the replication of the traditional seed dehulling process under controlled laboratory conditions on the oxidative status (Peroxide value, *p*-anisidine value, Totox index) of ricinodendron oil.

		0 day		3 days		10 days	
	Market kernels	**Non-boiled seeds**	**Boiled seeds**	**No light**	**Light**	**No light**	**Light**
Peroxide value ($meqO_2/kg$)	27.89 ± 0.40	0.69 ± 0.24^{e}	0.95 ± 0.14^{e}	3.78 ± 0.19^{d}	6.08 ± 0.15^{c}	9.19 ± 0.58^{b}	10.09 ± 0.54^{a}
***p*-anisidine value**	87.13 ± 1.40	2.84 ± 0.27^{c}	3.86 ± 0.62^{c}	9.51 ± 5.19^{bc}	11.16 ± 3.42^{bc}	28.10 ± 2.39^{a}	22.53 ± 1.22^{a}
Totox index	142.90 ± 0.54	4.22 ± 0.69^{c}	5.76 ± 0.77^{c}	17.06 ± 5.10^{b}	23.33 ± 3.24^{b}	46.48 ± 3.11^{a}	42.71 ± 2.13^{a}

With the exception of market kernel oil, serving as positive control for traditional dehulling, all oil samples originated from Bassila 1 seeds. Seeds were either dehulled directly, without any prior treatment (non-boiled seeds), or dehulled via water boiling (boiled seeds). Kernels from boiled seeds were dried in an incubator at 35°C, with or without light exposure, for 3 or 10 days. All oil samples were obtained by mechanical pressing of the kernels. Values are presented as mean ± SD (n = 3). No common superscript lowercase letter indicates a significant difference ($p \leq 0.05$) within each line.

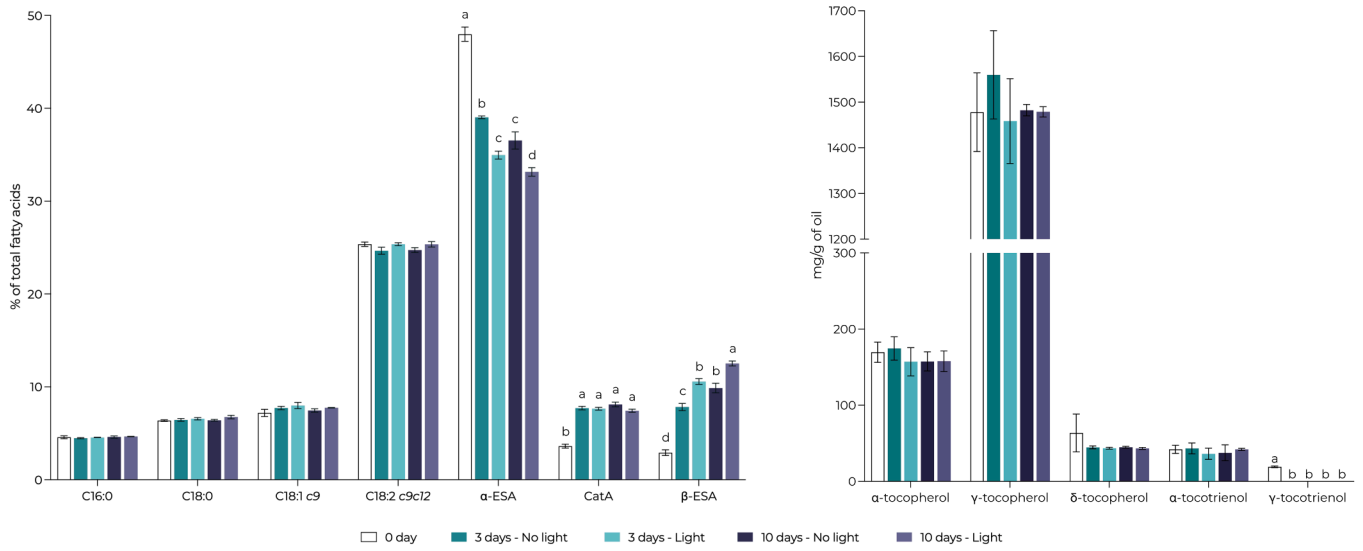

Fig. 2. Impacts of the replication of the traditional dehulling process under controlled laboratory conditions on the fatty acid and tocopherol-tocotrienol compositions of ricinodendron oil. α-ESA = α-eleostearic acid; β-ESA = β-eleostearic acid; CatA = catalpic acid. α-ESA, β-ESA, CatA are all expressed in α-ESA equivalents. No common superscript lowercase letter indicates a significant difference ($p \leq 0.05$) within each molecule.

change was observed in total vitamin E content ($p > 0.05$), suggesting that endogenous tocopherols and tocotrienols may be insufficient to prevent oxidation of ricinodendron oil during traditional dehulling. This observation is consistent with the findings of Nikiema et al. (2019), who did not quantify significant tocopherol degradation following traditional ricinodendron seed dehulling.

While the total fatty acid content was not significantly affected by the traditional process reproduction, we observed a shift in CLnA isomer proportions. After 3 days of incubation, both CatA and β-ESA proportions had already increased significantly ($p \leq 0.05$), likely due to the isomerisation of α-ESA, whose proportion decreased accordingly. Light-exposed kernels exhibited a significantly greater increase in β-ESA ($p \leq 0.05$) and a more pronounced decrease in α-ESA ($p \leq 0.05$) compared to kernels incubated in the dark, whereas CatA proportion was not affected by light exposure. After 10 days of incubation, α-ESA levels in both light-exposed kernels and their counterparts kept in the dark had decreased further ($p \leq 0.05$) while β-ESA levels had increased correspondingly ($p \leq 0.05$). CatA levels remained constant from day 3 to day 10, in both conditions. Overall, the lowest proportion of α-ESA (33.2%) and highest proportion of β-ESA (12.5%) were obtained in light-exposed kernels after 10 days of drying. These results show that under factors such as heat and sunlight, α-ESA undergoes isomerisation, mostly in β-ESA. This assertion is supported by the work of Nikiema and colleagues who investigated the isomerisation of α-ESA in ricinodendron oil (Nikiema et al., 2019, 2022).

3.3. Geographical variability of ricinodendron oil across different communes of Benin

As compositional data on ricinodendron oil remain scarce in the literature, we report here the fatty acid, tocopherol–tocotrienol and phytosterol profiles of several ricinodendron oils obtained from directly dehulled kernels originating from different communes of Benin (Adja-Ouèrè, Bantè, Bassila 2, Kétou-Lama and Pobè). All oils presented in this section were extracted by Soxhlet extraction.

The oxidative status and the fatty acid, tocopherol-tocotrienol and phytosterol compositions of the analysed ricinodendron oils are presented in Table 3. All samples exhibited very low levels of primary and secondary oxidation products, with Totox values consistently below 5, confirming the protective effect of direct seed dehulling. No significant differences ($p > 0.05$) were observed between samples for PV, *p*-AV or Totox values. Fatty acid profiles were highly similar across all samples, with only minor variations. CLnAs represented on average 53.5% of total fatty acids, with oil from Bassila 2 being significantly richer ($p \leq 0.05$) than the other samples. In contrast, the oil from Bantè contained the lowest proportion of CLnAs, with 48.0%. The predominant CLnA isomer was α-ESA, accounting for an average of 40.9% of total fatty acids. Two other CLnAs were also detected: β-ESA and CatA, representing an average of 6.0% and 5.8%, respectively. Non-conjugated fatty acids accounted for around 45.4% of total fatty acids, primarily as LA, followed by oleic, stearic, and palmitic acids in decreasing order of abundance. Other fatty acids were detected only in trace amounts

Table 3
Oxidative status (Peroxide value, *p*-anisidine value, Totox index) and composition (fatty acids, tocopherol-tocotrienols and phytosterols) of ricinodendron kernel oils obtained from seeds collected in several communes of Benin (Adja-Ouèrè, Bantè, Bassila 2, Kétou, Pobè).

		Adja-Ouèrè	Bantè	Bassila 2	Kétou	Pobè	Nikiema et al., 2019
Oxidative status	**Peroxide value** ($meqO_2/kg$)	1.82 ± 0.24[a]	1.72 ± 0.49[a]	1.24 ± 0.45[a]	1.56 ± 0.55[a]	1.56 ± 0.08[a]	—
	***p*-anisidine value**	0.45 ± 0.63[a]	0.53 ± 0.11[a]	2.15 ± 1.49[a]	1.00 ± 0.55[a]	0.10 ± 0.18[a]	—
	Totox index	4.33 ± 0.96[a]	3.96 ± 0.98[a]	4.62 ± 1.92[a]	4.12 ± 0.55[a]	3.22 ± 0.14[a]	—
Fatty acids (% of total fatty acids) (*w/w*)	**C16:0**	6.23 ± 0.12	4.25 ± 0.22	4.42 ± 0.02	6.16 ± 0.05	5.89 ± 0.10	3.8
	C18:0	6.58 ± 0.31	7.33 ± 0.37	6.06 ± 0.01	6.48 ± 0.06	6.36 ± 0.06	5.1
	C18:1 *c9*	5.04 ± 0.00	13.89 ± 0.70	7.24 ± 0.04	5.14 ± 0.05	5.79 ± 0.02	6.7
	C18:2 *c9c12*	27.92 ± 0.16	25.08 ± 1.27	21.47 ± 0.06	27.79 ± 0.22	27.75 ± 0.54	22.8
	C18:3 *c9t11t13* (α-ESA)	39.97 ± 1.28[b]	34.80 ± 3.15[c]	47.42 ± 0.57[a]	41.27 ± 0.86[b]	40.87 ± 0.34[b]	60.1
	C18:3 *t9t11c13* (CatA)	6.18 ± 0.25	6.27 ± 0.33	5.85 ± 0.21	5.70 ± 0.18	5.85 ± 0.17	1.1
	C18:3 *t9t11t13* (β-ESA)	6.30 ± 0.51	6.18 ± 0.34	5.39 ± 0.31	5.64 ± 0.36	5.67 ± 0.44	—
	Total CLnAs	53.27 ± 0.52[b]	48.04 ± 2.64[c]	59.52 ± 0.06[a]	53.40 ± 0.40[b]	53.15 ± 0.69[b]	61.2
Tocopherols-tocotrienols (mg/kg)	**α-tocopherol**	65.34 ± 4.18	147.54 ± 16.53	173.35 ± 11.54	76.62 ± 6.17	117.74 ± 2.77	140.86
	γ-tocopherol	1145.24 ± 23.44	954.47 ± 20.75	879.19 ± 44.78	1221.93 ± 34.36	1185.86 ± 51.70	1602.92
	δ-tocopherol	18.76 ± 1.12	40.37 ± 1.67	38.36 ± 2.74	19.10 ± 1.58	22.72 ± 1.16	44.58
	α-tocotrienol	10.00 ± 1.74	8.59 ± 0.32	9.11 ± 1.94	9.86 ± 0.55	19.85 ± 2.48	—
	γ-tocotrienol	28.35 ± 0.86	10.21 ± 1.21	10.33 ± 0.29	28.03 ± 1.22	27.92 ± 0.89	—
	δ-tocotrienol	N.D.	N.D.	N.D.	N.D.	N.D.	—
	Total	1267.69 ± 27.21[ab]	1162.18 ± 30.97[bc]	1110.35 ± 53.56[c]	1355.54 ± 41.21[a]	1374.10 ± 49.93[a]	1783
Phytosterols (mg/kg)	**Brassicasterol**	N.D.	N.D.	N.D.	N.D.	N.D.	—
	Campesterol	287.95 ± 16.87	331.45 ± 16.41	321.47 ± 18.01	274.36 ± 38.42	217.62 ± 53.17	289.8
	Stigmasterol	164.56 ± 11.87	177.10 ± 8.51	176.51 ± 9.05	167.01 ± 23.07	157.74 ± 33.62	116.73
	β-sitosterol	3274.74 ± 58.23	3344.67 ± 146.25	3197.73 ± 141.75	3119.97 ± 476.49	2489.98 ± 551.73	3368.93
	Total	3727.25 ± 85.74[a]	3853.22 ± 170.85[a]	3695.71 ± 168.65[a]	3561.33 ± 537.83[a]	2865.34 ± 638.4[a]	4025

All seeds were dehulled directly, without prior treatment, and oils were obtained by Soxhlet extraction. Data from Nikiema et al., 2019 are displayed for comparison purpose. Values are presented as mean ± SD (n = 3). N.D. = not detected; α-ESA = α-eleostearic acid; β-ESA = β-eleostearic acid; CatA = catalpic acid; CLnAs = conjugated linolenic acids. CLnAs (α-ESA, β-ESA, CatA) are all expressed in α-ESA equivalents. No common superscript lowercase letter indicates a significant difference ($p \leq 0.05$) within each line. Statistical comparisons are presented only for the oxidative status and contents in α-ESA, total CLnAs, total tocopherols-tocotrienols and total phytosterols.

(<1%). All analysed oils contained the same forms of vitamin E. Tocopherols were predominantly present in the form of γ-tocopherol, accounting for an average of 86% of total vitamin E, followed by α- and δ-tocopherol. Tocotrienols were detected at lower concentrations, as α- and γ-tocotrienol. No δ-tocotrienol was detected in any of the samples. Total tocopherol-tocotrienol contents of Adja-Ouèrè, Kétou and Pobè kernel oils were significantly higher ($p \leq 0.05$) than those of the other oil samples. Three different phytosterols were detected in all samples: β-sitosterol, campesterol, and stigmasterol, in order of decreasing concentration. Total phytosterol concentration was approximately 3540.57 mg/kg, with no significant difference between oil samples ($p > 0.05$). Overall, these results indicate that the composition of ricinodendron oil — in terms of fatty acids, tocopherols and phytosterols — is relatively conserved across different communes of Benin. Although slight variations were observed, the relative abundance of the major compounds remained consistent among samples. To our knowledge, only Nikiema et al. (2019) have analysed fatty acids, tocopherols, and phytosterols in ricinodendron oil extracted from manually dehulled kernels. Their results broadly align with ours in terms of the relative abundance of major compounds. Differences are to be noted concerning the CLnA composition: Nikiema and colleagues reported only limited amounts of β-ESA and no detectable CatA whereas our data revealed greater isomeric diversity. Other studies have focused solely on the fatty acid profile of traditionally processed kernels. They consistently report approximately 50% α-ESA and 50% non-conjugated fatty acids (LA, oleic acid, stearic acid, palmitic acid) (Assanvo et al., 2015; Ngo Njembe et al., 2021; Tchankou Leudeu et al., 2009). To note, none of those studies detected or quantified β-ESA or CatA. Nevertheless, comparison between our data and other works remains speculative, as the oxidation status of the analysed ricinodendron oils are often not available.

The present study has certain limitations, which also highlight directions for future research. First, the use of hot extraction methods may have affected the composition of ricinodendron oil, particularly regarding thermo-sensitive minor constituents such as polyphenols (Klisović et al., 2024). Although no major oxidative artefacts were observed following either chemical or mechanical hot extraction, future studies specifically focused on ricinodendron oil minor constituents should preferably rely on cold mechanical extraction methods to better preserve the native oil composition. In addition, the laboratory-controlled reproduction of the traditional dehulling process performed in this work necessarily simplifies real-life practices and only partially simulates environmental conditions, particularly sunlight exposure. This limitation is exacerbated by the lack of information regarding the provenance, storage duration and handling conditions of market kernels, preventing a precise attribution of the observed oxidation levels to the specific steps of the traditional dehulling process. Another limitation relates to the assessment of oxidative status. Although PV and *p*-AV are widely used indicators, recent studies suggest that they may not fully capture the specific oxidation pathways of CLnA-rich oils, which generate atypical oxidation products such as peroxide polymers or conjugated dienals (Do et al., 2023). Future work should therefore integrate more advanced analytical approaches, including size-exclusion chromatography for polymer analysis (Caldwell et al., 2011; Dobarganes et al., 2000) and LC–MS/MS for the identification and quantification of aldehydic compounds (Douny et al., 2016), as well as investigate their potential toxicity for human health. Finally, while this study focused on kernel processing, the oxidative stability of ricinodendron oil after extraction also warrants investigation, particularly regarding the use of protective strategies such as natural antioxidants (Nain et al., 2021).

4. Conclusion

Ricinodendron seeds are traditionally dehulled through water boiling followed by sun-drying. To our knowledge, the effects of this traditional treatment have never been investigated with regard to oxidation, despite the particularly high CLnA content of ricinodendron oil calling for caution regarding the use of pro-oxidative processes. In the

present work, we quantified elevated amounts of both primary and secondary oxidation products in kernels purchased on the Dantokpa market in Cotonou (Benin), far exceeding the recommended thresholds. We demonstrated the link between these markers and the traditional dehulling process, by performing a reproduction of the key steps of this process under controlled laboratory conditions. In contrast, oils obtained from kernels dehulled without prior treatment exhibited minimal oxidation levels, underscoring the pivotal role of sun-drying and heat in altering the oil's oxidative status. Our results warrant further investigation on traditionally processed kernels from different regions of West and Central Africa, given the potential public health implications that might be associated with the consumption of such highly oxidised products.

In addition, we analysed the composition of ricinodendron oils extracted from directly dehulled kernels collected across several communes of Benin, therefore addressing a current gap in the literature. All analysed oils contained high proportions of CLnAs, predominantly in the form of α-ESA, along with notable amounts of β-ESA and CatA. Different forms of vitamin E and phytosterols were also quantified, primarily γ-tocopherol and β-sitosterol, respectively. Only minimal compositional differences were observed among the extracted oils regarding fatty acids, vitamin E, and phytosterols, suggesting a certain homogeneity in the lipid composition of ricinodendron kernels across Benin.

CRediT authorship contribution statement

Antoine Ide: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Sarah Vellemans:** Writing – review & editing, Methodology, Conceptualization. **Flora Josiane Chadare:** Writing – review & editing, Resources. **Pui Yue Ho:** Investigation. **Aurélien Warnant:** Methodology. **Éric Mignolet:** Methodology. **Joëlle Quetin-Leclercq:** Writing – review & editing. **Achille Assogbadjo:** Resources. **Cathy Debier:** Writing – review & editing, Conceptualization. **Olivier Feron:** Writing – review & editing, Supervision. **Yvan Larondelle:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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