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Article

# Thread-Based Bienzymatic Biosensor for Linoleic Acid Detection

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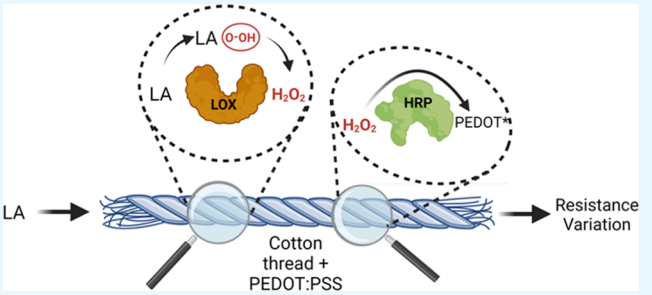
**ABSTRACT:** The concentration of nonesterified fatty acids (NEFAs) in biological media is associated with metabolic and cardiovascular disorders (e.g., diabetes, cancer, and cystic fibrosis) and in food products is indicative of their quality. Therefore, the early identification of NEFAs is crucial for both medical diagnosis and food quality assessment. However, the development of a portable and scalable sensor capable of detecting these compounds at a low cost presents challenges due to their considerable chemical and physical stability. This research endeavors to illustrate the viability of detecting linoleic acid using a chemiresistive bienzymatic sensor constructed with cotton thread. The sensor's design incorporates the conductive polymer poly(3,4-ethylenedioxythiophene):polystyrenesulfonate (PEDOT:PSS) within the thread, alongside the enzymes horseradish peroxidase (HRP) and lipoxygenase (LOX). By implementing this technology, a sensitive detection range spanning from 161 nM to 16.1 $\mu$M is achieved when the PEDOT:PSS/HRP/LOX system is integrated into a single thread. The sensor exhibits exceptional selectivity toward linoleic acid, owing to the specific enzymatic reaction between LOX and linoleic acid. This selectivity is upheld even in the presence of other unsaturated fatty acids. This system can be used for future designs with the capability to detect polyunsaturated fatty acids and other intricate biomolecules.




## 1. INTRODUCTION

Fatty acids (FAs) are aliphatic organic molecules of different lengths, usually with backbones formed by 4 to 28 carbon atoms and characterized by at least one carboxylic group −COOH.[1] FAs play different roles in the human body, such as structural units or energy suppliers.[2] It has been shown that there is a correlation between different metabolic diseases (e.g., cancer, diabetes, cardiovascular, and neurological) and the concentration of the nonesterified form of fatty acids (NEFAs) in body fluids such as plasma, urine, and saliva.[2−6] For example, detecting NEFAs concentration in urine is critical for early and rapid diagnosis of thyroid cancer.[5] Similarly, the concentration of linoleic acid in saliva is correlated to cystic fibrosis, the formation of calculi, and other diseases.[6,7] Hence, the identification of these compounds can contribute to the timely detection of these diseases. Moreover, the assessment of fatty acid (FA) concentration profiles in food items containing fat can be employed to ascertain the quality of certain food commodities, such as milk and olive oil. For instance, the concentration of FAs serves as an indicator of milk freshness,[8] as well as the provenance and processing of olive oil.[9]

FAs are currently quantified by spectroscopic techniques such as gas or liquid chromatography.[10] Such methods are associated with high capital and operating costs as they require specialized equipment, professional staff for operation, and long analysis time. It would be ideal to develop a rapid and cost-effective technique for *in situ* FA detection. One of the challenges in the detection of FAs, more specifically NEFAs, is their relative stability due to the long carbon chain in their backbone. Different methods have been attempted for their rapid detection, such as using click-chemistry to tune oligonucleotides to trigger a fluorescent response to oleic acid between 2 $\mu$M and 14 $\mu$M,[11] the acyl-coenzyme A pathway to enzymatically detect oleic acid,[12] or even G-protein coupled receptors that can detect medium-chain fatty acids in a whole cell system.[13] Although these methods have demonstrated effectiveness in detecting FAs, they are not yet accessible in the commercial market. This is primarily due to the significant hurdles associated with their mass production, especially in the context of creating portable apparatus. Furthermore, there have been advancements in colorimetric sensors designed to identify NEFAs in food products.[14] However, it is worth noting that this approach might not be suitable for applications in diagnostics and medical fields where precise quantitative analysis is essential.[15]

Here, we introduce a simple and scalable electrochemical sensor for the rapid detection of NEFAs based on multiple enzymatic reactions. To this end, linoleic acid (LA) was selected as a model NEFA, and we assessed the feasibility of

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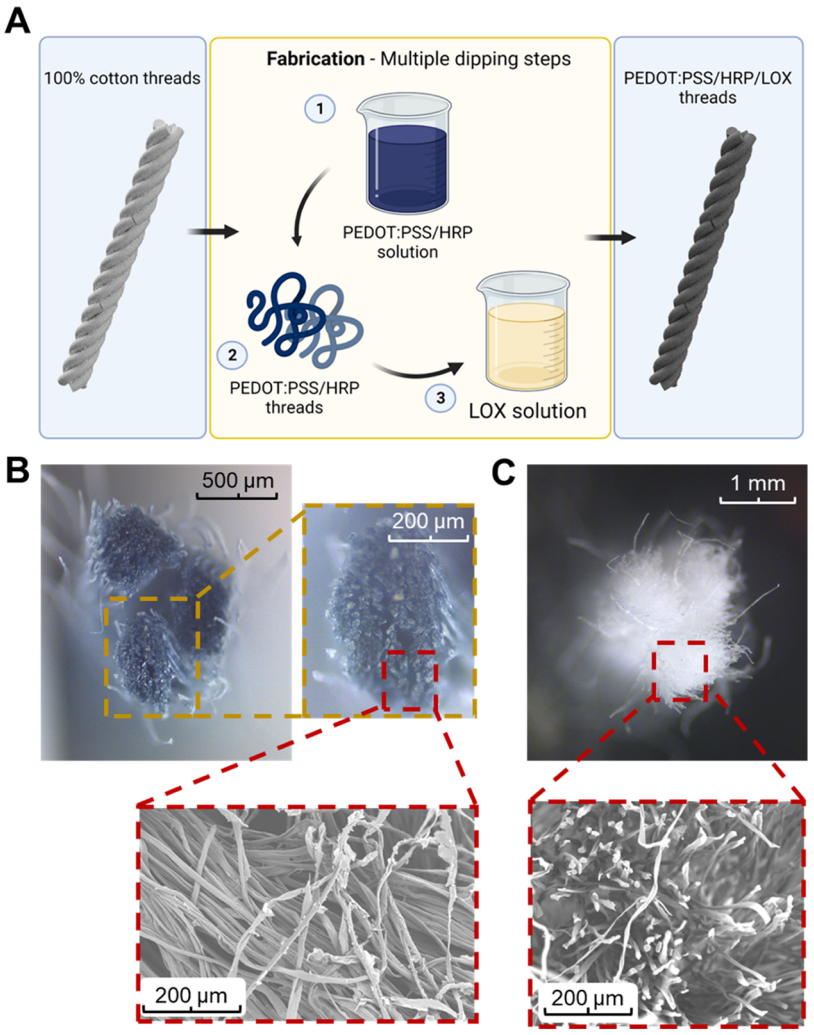


**Figure 1.** (A) Schematic depiction of the dipping procedure to produce the PEDOT:PSS/HRP/LOX threads. (B) Optical microscope and SEM image of the cross-section of a PEDOT:PSS/HRP-treated thread. Notice how the dark blue color typical of PEDOT:PSS is also visible in the core of the filaments, and polymer fragments are visible along the thread strands in the SEM image (C) Optical microscope and SEM image of an untreated thread for comparison.

using subsequent enzymatic reactions via lipoxygenase (LOX) followed by horseradish peroxidase (HRP) that are embedded in a conductive polymer for measuring linoleic acid analyte quantitatively. To miniaturize the sensor and reduce sample size, these enzymes and poly(3,4-ethylenedioxythiophene):polystyrenesulfonate (PEDOT:PSS) are embedded in cotton threads. Our previous studies illuminated that PEDOT:PSS/HRP embedded in porous substrates was an efficient system for the quantitative detection of hydrogen peroxide ($H_2O_2$) using electrochemical measurements.[16,17] Hence, this system may prove suitable for coupling with LOX, an enzyme that catalyzes the formation of hydrogen peroxide from polyunsaturated fatty acids (PUFAs).[18,19] To the best of our knowledge, this is the first time that an electrochemical biosensor based on the catalytic effect of LOX has been designed based on multiple enzymatic reactions in the solid phase for the quantitative detection of fatty acids.

## 2. MATERIALS AND METHODS

**2.1. Materials.** Highly conductive PEDOT:PSS aqueous dispersion (1.1 wt % in water), peroxidase from horseradish (198.6 units $mg^{-1}$ solid), hydrogen peroxide (30 wt %), arachidonic acid (>95%), lipoxidase (or lipoxygenase) from *Glycine max* (soybean) (>50,000 units $mg^{-1}$ solid), and linoleic acid >99% were purchased from Sigma-Aldrich (Merk, Australia). Oleic acid (1 mEq) was purchased from Wako as part of the Wako NEFA kit. Milford Soft 100% cotton threads ($d$ = 1.03 mm ± 0.01 mm, 5 twists per inch) were purchased from Spotlight (Australia). Milli-Q water and ethanol (EtOH) (95%v in Milli-Q water) were used to prepare the solutions.

**2.2. Preparation of Threads.** Different amounts of HRP ranging from 0 to 5 mg $mL^{-1}$ were dissolved in Milli-Q water and then mixed with a stock solution of PEDOT:PSS to obtain a final solution with a 2:3 volume ratio of HRP and PEDOT:PSS solutions, as per our previous work.[16,17] The solution was stirred gently for 5 min to avoid any bubble formation. Cotton threads with 14 cm length were submerged in 20 mL of the PEDOT:PSS/HRP solution and were kept at 4 °C for 2 h to ensure the complete absorption of the solution into threads while maintaining the enzyme activity. Then the threads were dried at 37 °C for 90 min. Finally, a 10 cm length of thread was used by cutting 2 cm from each side. These threads were used for the next stage of the experiment. A


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microscope (Leica DM1000) was utilized to take the optical images of the threads.

**2.3. Sensing Measurements.** The initial resistance of the dry thread was measured. Then, 40 $\mu$L of analyte solution—either hydrogen peroxide or linoleic acid—was added to the central section of the thread. This amount is enough to wet most of the thread, without overflowing. The thread was then kept at room temperature for 90 min to dry before the final resistance was recorded. The performance of the sensor was assessed by calculating the resistance variation in percentage between the value before and after exposure to the analyte. The procedure was repeated with various threads and for different concentrations of the analyte. No electrolyte was utilized. The resistance was recorded with a Keithley 2450 potentiostat which was used as a 2-wire ohmmeter.

**2.4. UV−visible Absorption.** HRP dissolved in Milli-Q water (4 mg mL$^{-1}$) was mixed with 540 $\mu$L of a PEDOT:PSS solution following a volume ratio of 2 to 3 between the respective chemicals. Then, 100 $\mu$L of water or hydrogen peroxide in different concentrations was added into the PEDOT:PSS/HRP solution. The solutions were gently stirred before being diluted (40×) with Milli-Q water. Similarly, 700 $\mu$L of a PEDOT:PSS/HRP solution was mixed with 300 $\mu$L of a LOX-LA solution. The latter was obtained by mixing 9 parts in volume of a LOX solution in Milli-Q water (3 mg mL$^{-1}$) and one part linoleic acid in different concentrations. The solution, after being stirred, was then diluted. From these solutions, 200 $\mu$L were sampled, and each was added to a 96-well plate for UV/Visible (UV−vis) absorption analysis. The absorption spectra between 400 and 1000 nm were recorded with a SpectraMax M3 spectrophotometer.

**2.5. Scanning Electron Microscopy (SEM).** Threads were coated with an 8 nm layer of gold via plasma sputtering before being analyzed using a scanning electron microscopy unit (PhenomTM XL G2 Desktop SEM, Thermo Fisher Scientific) with a back scattered electron detector (15 kV).

**2.6. Data Analysis.** The statistical studies have been conducted via GraphPad Prism (v9.4.1) on data consisting of at least 3 repetitions, of which average and standard deviation are shown. Error bars in the graphs represent the standard deviation.

## 3. RESULTS AND DISCUSSIONS

**3.1. Fabrication and Validation.** Our prior study demonstrated that PEDOT:PSS/HRP-based systems exhibited sensitivity in detecting various concentrations of hydrogen peroxide in both gaseous and liquid phases.[16,17] For example, the previously developed PEDOT:PSS/HRP paper-based sensor demonstrated a linear detection range spanning from 61.3 nM to 613 $\mu$M.[17] In this study, cotton threads were selected as the sensor support material. The preparation of thread samples involved a two-step process: first, immersing cotton threads in a PEDOT:PSS/HRP solution, followed by immersion in a LOX solution, as illustrated in Figure 1A. Before employing this setup, the optimal quantity of each enzyme required to yield a reasonable signal (measured as resistance variation or $\Delta R$) was determined (Figure S2). It was ascertained that utilizing a 4 mg mL$^{-1}$ HRP solution for constructing the samples resulted in a $\Delta R$ change proportional to the quantity of $H_2O_2$ introduced ($\Delta R \approx -20\%$ for 16.1 mM, as depicted in Figure S1). This approach also ensured an appropriate wicking rate and even dispersion of the PEDOT:PSS/HRP ink throughout the threads (as shown in Figure 1B,C), with PEDOT:PSS visible along the thread strands in the SEM image (see also Figure S3), albeit causing an increase in sensor resistance to approximately 40 k$\Omega$ compared to lower enzyme concentrations (as indicated in Figure S4).

This phenomenon was described in detail in our previous study.[17] Briefly, HRP catalyzes the reduction of $H_2O_2$ while interacting and exchanging electrons with PEDOT:PSS. This effect results in PEDOT:PSS oxidation, which in turn leads to increasing bipolaron levels, hence increasing conductivity.[17,20] The interaction between HRP and PEDOT:PSS changes PEDOT unit conformation, known as quinoid (more conductive) and benzoid (less conductive).[21] When HRP reduces hydrogen peroxide in low quantities, i.e., below hundreds of $\mu$M, it favors the transition of PEDOT from benzoid to quinoid, lowering the resistance of the system. On the other hand, an excessive amount of $H_2O_2$ leads to the degradation of PEDOT:PSS.[16,17]

**3.2. Linoleic acid detection via bienzymatic reaction.** Following the validation of the PEDOT:PSS/HRP system, we assessed the feasibility of detecting fatty acids for the first time via a bienzymatic reaction using a thread-based sensor. To this end, LOX was embedded into the thread for the detection of LA as a model PUFA. LOX is an iron-containing enzyme that catalyzes the formation of hydroperoxide derivatives starting from PUFAs.[19] It is a relatively unstable enzyme compared to HRP, particularly in acidic conditions. In fact, the introduction of LOX along with HRP into the PEDOT:PSS acidic solution resulted in the creation of a thread-based sensor that did not exhibit any response to variations in LA concentration (as depicted in Figure S5A). Furthermore, when the sensors were loaded solely with either HRP or LOX, no evident correlation was observed between LA concentration and measured response (see Figure S5B). Consequently, for the fabrication of the sensor, PEDOT:PSS/HRP threads were immersed for 1 h in a solution containing 3 mg mL$^{-1}$ of LOX before being dried at room temperature (see Figure S6).

The PEDOT:PSS/HRP/LOX sensor showcased a discernible reduction in resistance as the concentration of LA increased, up until 16.1 $\mu$M LA ($\Delta R = -23.6\% \pm 5.7$). Beyond this concentration threshold, the resistance started rising, as demonstrated in Figure 2A. This observed phenomenon was attributed to the relatively limited solubility of LA in water (approximately in the micromolar range, as previously reported by Khuwijitjaru et al.)[22] and the presence of a nonhomogeneous emulsion within this specific concentration range. The sensor exhibited a semilogarithmic linear response to LA from 80.5 nM to 16.1 $\mu$M (Figure 2A). The lowest concentration which could be measured was 161 nM and various values of the limit of detection have been calculated and reported in Table S1. Furthermore, the semilogarithmic calibration curve trend used resembles an enzymatic kinetic reaction trend, where the signal initially rapidly increased before approaching a plateau once surpassed a certain LA concentration. However, the bienzymatic system employed rendered the kinetics of the reaction more complex and hence reduced the similarity with a single-enzyme-catalyzed reaction kinetic (more information in the SI − Figure S7).

*3.2.1. The detection mechanism.* The detection mechanism of this bienzymatic thread-based sensor is governed by subsequent enzymatic reactions. LOX catalyzes the incorporation of a hydroperoxide group at the double bond site within


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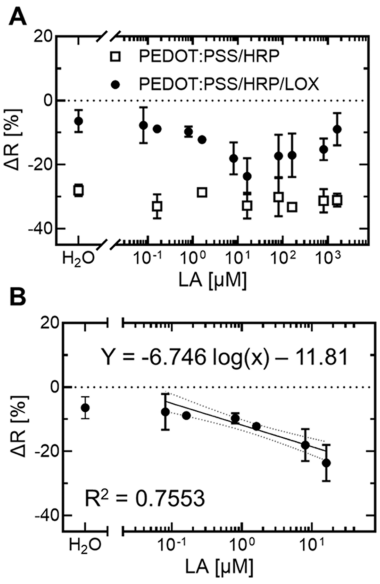


**Figure 2.** (A) Percentage of resistance variation for PEDOT:PSS/HRP/LOX sensor produced through two distinct dipping steps compared with the PEDOT:PSS/HRP sensor. The error bars remain negligible when the concentration of LA is less than 10 $\mu$M (refer to Table S2). (B) Inset of graph 2A, representing the detection range and the semilogarithmic linear regression line and equation. The $R^2$ is also reported.

a pentadiene segment of the primary backbone found in polyunsaturated fatty acids (PUFAs) − in this specific instance, linoleic acid.[19] During its oxidation, LOX modifies the valency of the iron atom situated within its core.[23] Within a complete enzymatic cycle of fatty acid degradation, the resulting hydroperoxide fatty acid interacts with other enzymes, causing the breakdown of the backbone into smaller fragments.[23] In the absence of the enzymes upstream in the process, the unstable hydroperoxide group eventually dissociates from the primary fatty acid backbone through either a redox reaction or the generation of radicals due to the cleavage of the peroxide bond. The occurrence of reactive oxygen, hydrogen, and hydroxyl species subsequently facilitates the generation of hydrogen peroxide within the sample solution. Although certain molecules may form stable compounds before reaching the PEDOT:PSS/HRP system, it is hypothesized that hydrogen peroxide eventually reaches horseradish peroxidase (HRP), initiating an interaction between PEDOT:PSS and HRP. Consequently, this interaction induces a change in resistance by prompting a shift to either the benzoid or quinoid form of PEDOT.[17] The proposed mechanism is schematically represented in Figure 3A.

UV−vis absorption spectroscopy was conducted to confirm the impact of linoleic acid on the PEDOT:PSS spectra in the presence of both enzymes. The absorption spectrum of PEDOT:PSS displays a supplementary peak around 900 nm, the intensity of which is directly proportional to the concentration of bipolarons present within the system.[24] As discussed in our previous publication, an elevation in the bipolaron concentration and, consequently, in peak intensity would suggest an enhancement in the conductivity of PEDOT:PSS.[17] Analogous to observations in our prior study, the introduction of low concentrations of hydrogen peroxide (e.g., 16.1 $\mu$M) into a PEDOT:PSS/HRP solution led to a marginal increase in peak intensity, indicating heightened conductivity. Conversely, the inclusion of higher levels of hydrogen peroxide (e.g., 16.1 mM) resulted in a decrease in peak intensity (Figure 3B).[16,17] Similarly, the introduction of

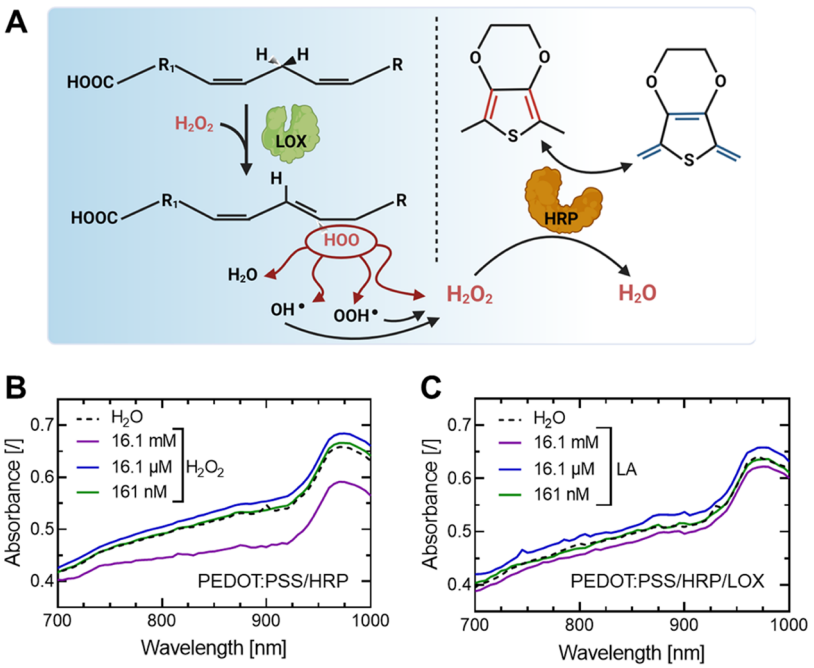


**Figure 3.** (A) Schematic depiction of the proposed LA detection mechanism of a thread-based PEDOT:PSS/HRP/LOX sensor. From the left, LOX insert a hydroperoxide group at the end of the pentadiene moiety, which is then released in various forms, leading to the formation of hydrogen peroxide which is then used by HRP to enable to switch in PEDOT conformation between benzoid and quinoid. (B) UV−visible absorption spectra of PEDOT:PSS/HRP after the addition of different amounts of hydrogen peroxide and (C) PEDOT:PSS/HRP/LOX after the addition of different levels of linoleic acid (left).


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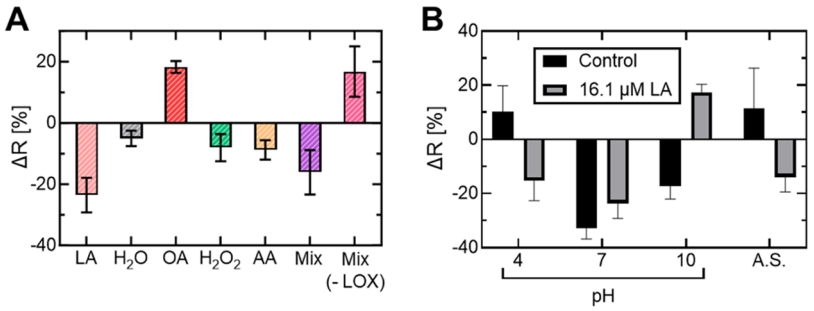


**Figure 4.** (A) Variation in the percentage of resistance after the addition of different compounds. Mix: a mixture of LA, OA, and AA (all at 16.1 $\mu$M), Mix (- LOX): FAs mixture when used on a sensor without LOX. (B) Variation of resistance after the addition of 16.1 $\mu$M LA dissolved in water solutions of different pH and in artificial saliva (A.S.). The control results are obtained from sensors exposed to the sole solvent (i.e., no LA is present in solution).

low concentrations of linoleic acid caused a slight rise in peak intensity, whereas higher linoleic acid concentrations brought about a decline in peak intensity. Although these effects were less pronounced when utilizing PEDOT:PSS/HRP/LOX and linoleic acid, it can still be inferred that alterations occurred within the PEDOT:PSS structure (Figure 3C). Additional data, including complete absorption spectra, are presented in the supplementary document (Figures S8 and S9).

*3.2.2. Selectivity.* The selectivity of the bienzymatic sensor toward LA was assessed by adding interferents, and the results are presented in Figure 4A. Oleic acid (OA) and arachidonic acid (AA) were selected as examples of monounsaturated and polyunsaturated fatty acids, respectively. Furthermore, the effect of $H_2O_2$ was also studied, as it could bypass the bienzymatic system and cause a reduction in resistance by simply reacting with HRP.

Contrary to LA, the addition of OA resulted in an 18.24% ± 1.91% increase in the resistance. As OA is a monounsaturated fatty acid, it can hardly react with LOX. The observed increase in resistance is caused by the electrical insulating nature of OA. On the other hand, AA decreased the resistance, although not to the same extent as achieved by LA. This effect was most likely attributed to the structural similarity between AA and LA. The presence of a pentadiene moiety in both compounds—even though in different locations of the carbon chain, allowed LOX to react weakly with AA. Finally, the addition of $H_2O_2$ led to a decrease in resistance. The decrease was lower than the one caused by LA and by the one measured in PEDOT:PSS/HRP samples. This suggests either a partial inactivation of HRP or a steric hindrance effect from LOX.

Furthermore, the sensor was used to analyze a mixture solution of LA, OA, and AA (16.1 $\mu$M of each) in the presence and absence of LOC ("Mix" and "Mix (- LOX)" in Figure 4, respectively). The sensors including LOX showed a decrease in resistance slightly smaller compared with the one caused by LA, albeit not significantly smaller ($p = 0.6352$), indicating the possibility of employing this sensor even in complex environments. On the other hand, the same solution increased the resistance of a PEDOT:PSS/HRP sensor (Mix (- LOX) in Figure 4): as LOX was not present to catalyze the degradation of LA, the presence of fatty acids just lowered the conductivity of the system. This opens the possibility of selectively detecting LA in complex mixtures, potentially coupling an active (PEDOT:PSS/HRP/LOX) and a passive (PEDOT:PSS/HRP) sensor to improve selectivity by calibrating the system based on both their signals.

The necessity of an active-passive sensing design was further substantiated when the PEDOT:PSS/HRP/LOX sensor was tested against LA solutions at varying pH levels (*i.e.*, 4, 7, and 10). As illustrated in Figure 4B, the sensor's response significantly changed upon the addition of basic or acidic solutions. This phenomenon is likely attributed to the pH-sensitive nature of PEDOT:PSS, which not only affects conductivity but may also influence the interaction between PEDOT:PSS and HRP. In fact, the conformation of PEDOT changes from quinoid to benzoid while increasing the pH,[33] which may alter the effect of the interaction and electron transfer with HRP. Moreover, exposing the enzymes to different pH levels may impede their catalytic activity. Additionally, the sensor was tested with LA dissolved in artificial saliva (A.S.), a water solution containing common salts found in human saliva (e.g., sodium chloride, potassium chloride, potassium thiocyanate, and urea). In this scenario, although the sensor's response differed from that in water, it remained possible to distinguish the solvent's effect from that of LA. Nonetheless, further research is imperative to evaluate the sensor's selectivity, accuracy, and stability, as well as to assess its performance with real samples such as urine and plasma.

**3.3. Comparison with the literature.** Various categories of amperometric sensors have been devised to detect fatty acids. As depicted in Table 1, these sensors require relatively intricate detection mechanisms involving antibodies,[25,26] layer-by-layer assembly,[27] specialized electrodes like dissolved oxygen probes,[28] and nanostructures.[29] In contrast, the miniaturized thread-based sensors produced in this study demonstrated a detection range comparable to previously developed sensors while exhibiting a lower limit of detection of approximately 1.61 $\mu$M. These sensors are also flexible, involve simple fabrication steps, and can be produced at a relatively modest cost. Contrary to other sensors in the literature, the PEDOT:PSS/HRP/LOX sensor reported here was designed as a single-use device, as the conductivity changes induced by exposure to LA are irreversible. Furthermore, it is crucial to note that the sensor exhibits optimal performance within 24 h following the adsorption of the LOX enzyme into the thread. Therefore, it is recommended to add the enzyme solution immediately prior to measurement and utilize the sensor within the first day.

Despite the fact that LOX does not exclusively bind to LA but rather to a group of polyunsaturated fatty acids (PUFAs), differentiation among them is achievable by selecting the specific enzyme from the LOX family (e.g., 15-Lipoxygenase


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preferentially catalyzes the degradation of arachidonic acid and LA)[30] and through active-passive sensing. Moreover, other PUFAs belonging to the same family, such as arachidonic acid, α-linolenic acid, and docosahexaenoic acid, exist in plasma and other biological media at significantly lower concentrations (i.e., two to 3 orders of magnitude lower).[31] Consequently, the sensor's response still enables precise quantification of LA. This is potentially applicable to different foods, given that each food possesses a distinct fatty acid profile. Furthermore, only a limited number of the listed sensors were employed for the detection of linoleic acid, underscoring the significance and relevance of the PEDOT:PSS/HRP/LOX sensor.

As depicted in Figure 5, the detection ranges of these binary enzymatic thread-based sensors are suitable for medical and

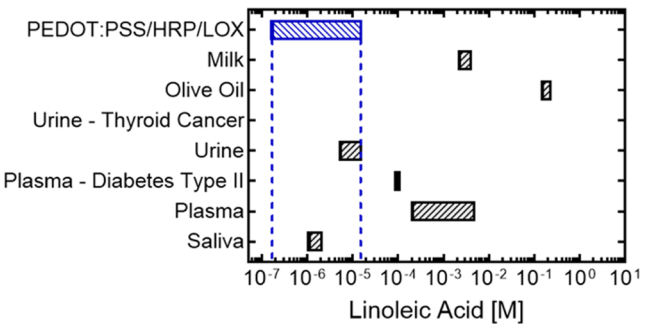


**Figure 5.** Common ranges of linoleic acid in body fluids and some fat-containing foods. The top bar (PEDOT:PSS/HRP/LOX) represents the detection range of the sensor developed in this study. The data for the concentration of LA in saliva,[7] plasma,[31,34] urine,[5] olive oil,[35] and milk[32] are taken from the literature.

diagnostic purposes. The detection range of the PEDOT:PSS/HRP/LOX sensor encompasses the concentration of linoleic acid commonly observed in human urine and saliva (Figure S10). Conversely, higher concentrations of LA, such as in the case of food or plasma, would require a different system of detection. It may be possible to tune the components concentrations or the system design to shift the detection range toward higher values (i.e., in the millimolar range), though further testing is required. It would be indeed beneficial to analyze LA in plasma, which serves as an indicator for various cardiovascular and metabolic disorders, including diabetes.[4,31] Similar prospects are plausible in the realms of the food industry and the assessment of milk quality.[32]

**3.4. Future perspectives.** The thread-based sensor developed within this study demonstrates favorable reproducibility for LA concentrations below 16.1 μM, as indicated by the minimal magnitude of error bars in Figure 2B. However, at higher concentrations, the standard deviation becomes more pronounced due to the partial solubility of LA in water. To address this limitation, the introduction of a solvent and surfactant holds the potential to enhance LA solubility and consequently broaden the range of detectable concentrations. The automation of the fabrication, as demonstrated by Alshabouna et al.,[36] and the introduction of better contacts could also improve reproducibility and accuracy. Nonetheless, as evidenced in Figure 4, the present detection range remains suitable for applications such as the quantification of LA in saliva and urine. It is hypothesized that optimizing the fabrication process by better control of parameters like thread

**Table 1. Comparisons of Non-esterified Fatty Acids Sensors from the Literature**[a,c]

| sensor | mechanism | analyte | detection range [μM] | LOD [μM] | response time | sample volume | refs |
|---|---|---|---|---|---|---|---|
| Co(II) phthalocyanine-MWCNTs - carbon paste | amperometric | stearic acid, oleic acid, linoleic acid, linolenic acid, and arachidonic acid | 26.7−712[b] | 8.9 | N.A. | N.A. | 29 |
| SPE/poly(dimethyldiallyammonium chloride)/MWCNTs/ACS/ACOD | amperometric | oleic acid | 400−1.2 × $10^3$ | 400 | N.A. | N.A. | 27 |
| SPE/rGO/EDC-NHS/NEFA antibody | amperometric | oleic acid | 100−5.0 × $10^3$ | 111 | N.A. | N.A. | 25 |
| SPE/$MoS_2$/NEFA antibody | amperometric | oleic acid | 100−5.0 × $10^3$ | N.A. | N.A. | N.A. | 26 |
| $[Ru(bpy)_3]^{2+}$-GO/LOX | amperometric | oleic acid | 100−1.0 × $10^3$ | 100 | 30 s | N.A. | 38 |
| DO probe/gelatin/LOX | amperometric | linoleic acid and α-linolenic acid | 12.8−160.5 | 12.8 | 5 min | N.A. | 28 |
| | | | 3.8−18.9 | 3.8 | | | |
| PEDOT:PSS/HRP/LOX | chemiresistive | linoleic acid | 0.161−16.1 | 0.161 | 90 min | 40 μL | this work |

[a]MWCNT: multiwall carbon nanotubes; SPE: Screen-printed electrode; ACS: acetyl-CoA synthetase; ACOD: acetyl-CoA oxidase; rGO: reduced graphene oxide; EDC: N-(3-(Dimethylamino)propyl)-N′-ethylcarbodimide hydrochloride; NHS: *N*-hydroxysuccinimide; NEFA: nonesterified fatty acids; bpy: bipyridyl; GO: graphene oxide; DO: dissolved oxygen probe. [b]Data refer to the detection of linoleic acid. [c]Only part of the range is linear.


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thickness, tension, pore dimensions, and analyte flow rate could significantly enhance reproducibility.[36]

Environmental conditions, such as temperature and humidity, constitute another factor that may influence the reproducibility and reliability of the sensor results. Specifically, temperatures beyond the 0−40 °C range can diminish enzyme activity, thereby introducing variability in the outcomes. Additionally, humidity has been demonstrated to impact the conductivity of PEDOT:PSS-based systems. This effect is primarily attributed to swelling, the hindrance of electron hopping by water molecules, and the formation of a meniscus at elevated relative humidity levels.[17,37] Consequently, further investigations are necessary to validate results under varying environmental conditions. Nonetheless, it is anticipated that the implementation of a passive-active system, as previously suggested, can enhance sensor reliability and mitigate false results.

When devising sensors involving biological compounds such as enzymes, determining the optimal storage conditions is significantly important. Previous reports indicate that the activity of both HRP and LOX can be maintained for up to two years when stored at −20 °C under dry conditions. Consequently, evaluating their performance under various storage conditions becomes crucial to ascertaining the shelf life of these thread-based sensors.

Sample size constitutes another critical consideration for prospective prototype development, particularly concerning the evaluation of sensor applicability across diverse scenarios. For example, it was observed that the thread-based sensor necessitates 40 μL of analyte for analysis. Furthermore, demonstrating its applicability to detect LA in different environments, the effect of biological media or different solvents on analysis needs to be assessed for calibration purposes.

## 4. CONCLUSIONS

In this study, we developed a low-cost and miniaturized device based on a bienzymatic reaction for detecting a complex molecule such as linoleic acid (LA). This sensor was fabricated from cotton thread and via impregnation with PEDOT:PSS and the enzymes LOX and HRP. In the presence of both enzymes in a single thread, the sensor exhibited a detection range between 161 nM and 16.1 μM LA, requiring only 40 μL of sample. The bienzymatic sensor system showed different responses toward various fatty acids (i.e., LA, OA, and AA), highlighting its potential application for the selective detection of this analyte in complex mixtures. This early study shed light on the field of chemiresistive sensors for the future design of miniaturized and portable devices for monitoring and detecting complex molecules rapidly. Further studies will be conducted to develop the prototype as a disposable point-of-care device for detecting PUFAs in various biological systems for diagnostics and monitoring food quality.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c07394.

Hydrogen peroxide detection; Figures S1−S10; and references (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

AA, arachidonic acid; ACOD, acetyl-CoA oxidase; ACS, acetyl-CoA synthetase; bpy, bipyridyl; EDC, N-(3-(Dimethylamino)-propyl)-N′-ethylcarbodimide hydrochloride; DO, dissolved oxygen probe; EtOH, ethanol; HRP, horseradish peroxidase; FA, fatty acids; GO, graphene oxide; LA, linoleic acid; LOX, lipoxygenase; MWCNT, multiwall carbon nanotubes; NEFA, nonesterified fatty acids; NHS, *N*-hydroxysuccinimide; NEFAs, nonesterified fatty acids; OA, oleic acid; PEDOT:PSS, poly(3,4-ethylenedioxythiophene):polystyrenesulfonate; PUFA, polyunsaturated fatty acids; rGO, reduced graphene oxide; SPE, screen-printed electrode



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