Evaluation of 3-MCPD content of commonly consumed food in Côte d'Ivoire using bimodal UV-Vis/electrochemistry technique

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ABSTRACT
3-monochloropropane-1,2-diol (3-MCPD) is known as one of the neo-formed contaminants widely distributed in foodstuffs, especially in processed foods with high sides effects for human health. In this study, 3-monochloropropane-1,2-diol (3-MCPD) content for eleven (11) different types of foods from local market were evaluated in two ways using: (1) cysteine modified AgNPs (Cys-AgNPs) as functional nanomaterials for UV-Vis technique, and (2) gold-modified by Cys-AgNPs (Cys-AgNPs/Au) in electrochemistry sensor. High concentrations of 3-MCPD were found in most studied samples namely in liquid foods (palm oil and frying oil from tuna fish), fried foods (fried ripe banana, roasted peanut, banana chips, fried tuna and potato fries), smoked mackerel and roasted foods (pork barbecue). The maximum 3-MCPD content were 1085 μg/kg in liquid foods, 582 μg/kg in fried foods, 156 μg/kg in smoked and 716 μg/kg in roasted foods. As exhibited, the level of 3-MCPD in food products on the Ivorian market is relatively higher. This study indicates the importance of monitoring 3-MCPD content in food products for both domestic and industrial processing.

Keywords: Colorimetric method, electrochemical method, cysteine modified silver nanostructure, food contaminant, neo-formed contaminant, 3-monochloropropanediol.

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INTRODUCTION

Thermal processing is one of the most important sterilization techniques to ensure food safety and quality (Houssou et al., 2015; Fouepe et al., 2016; Zhao et al., 2017). This thermal processing induces chemical reaction, particularly, Maillard reaction, which confers its attractive color, taste, aroma and texture. Although the appearance of these by-products plays an important role, including consumer interests in health, food quality, convenience and safety, other known as neo-formed contaminants (NFCs) are considered a public concern (Birlouez-Aragon et al., 2010; Capuano and Fogliano, 2011; Ayoade and Adegbite, 2016; Aristil, 2019). Consumer exposure to these neo-formed substances mainly acrylamide, 3-monochloropropanediol (3-MCPD), 3-MCPD esters, furan and its derivative can induce potential adverse effects (Zhao et al., 2017). Currently, only a limited number of these NFCs are subject of authorized regulations (Habermeyer et al., 2011). The chloropropanol isomers have attracted increasing attention because of their apparition in various foods which can be seen as source of human exposition. A survey led on animals has demonstrated that 3-MCPD and 1,3-Dichloropropan-2-ol (1,3-DCP) have carcinogenic effect (Genualdi et al., 2017). In addition, the administration of 3-MCPD studies in rats via drinking water for 2 years has shown an increase of the impact of renal adenomas, tumors of the cells of Leydig, mammary tumors among males (fibroadenomas), and adenomas and carcinomas of the foreskin (JECFA, 2018). Furthermore, the studies of short and long-term toxicity on the rodents have indicated the nephrotoxicity and the testicular toxicity of 3-MCPD after injection of the regular doses to rats. For these reasons, International Agency for Research on Cancer (IARC) has classified 3-MCPD as carcinogen (Group 2B) (Sadowska-Rociek, 2017), while Environmental Health Hazard Assessment (OEHHHA) has suggested 3-MCPD as carcinogenic substance, congenital malformations and other reproductive harm. Lately, the Committee of experts of Food and Agriculture Organization and World Health Organization (FAO/WHO) on the food additives (JECFA) has proposed to reduce the supportable daily dose (DJD) to 2 kg bw/day for 3-MCPD (Leigh and MacMahon, 2016; Yang et al., 2018). Moreover, these contaminants are known to be formed in various hydrolyzed protein products when hydrochloric acid is used in the treatment process (Genualdi et al., 2017). Their formation can also originate from glycerol or acylglycerols in the environment of chloride ions and can be influenced by a series of factors including moisture, lipid content, pH and food type (Sadowska-Rociek et al., 2018). With the above-mentioned concerns and their presence in large foods, it is necessary to set some techniques to detect these NFCs to protect human health from their adverse effects by avoiding their occurrence. In view of the risks that the presence of 3-MCPD in foods can induce for human health, a number of risk management strategies have been intensified, including the establishment of a maximum level of 0.02 mg/kg for liquid products containing 40% dry matter by the European Commission (EC, 2006) and the development of a code of practice by the Codex Alimentarius Commission (CAC, 2008). In addition, a monitoring plan for the presence of 3-MCPD in food has recently been recommended to EU’s Member (Younes et al., 2021). The methods described in the literature for the determination of 3-MCPD usually employ gas chromatography-mass spectrometry (GC-MS) (Zhou et al., 2014; Dubois et al., 2019) and require a derivatization step to enhance volatility and sensitivity. The most common derivatization reagents are boronic derivatives (e.g. phenylboronic acid), heptafluorobutyryl derivatives (e.g. heptafluorobutyrylimidazole) and dioxolane derivatives (Wenzl et al., 2007; Baer et al., 2010). Other procedures report the use of liquid chromatography-mass spectrometry (LC-MS/MS) (Custodio-Mendoza et al., 2019), attenuated total reflection Fourier transform infrared spectroscopy (Wong et al., 2019), high performance liquid chromatography (HPLC)-
ultraviolet detection (Hui-Ying et al., 2018) or high-performance liquid chromatography (HPLC) coupled to fluorescence detection as alternatives to GC-MS methods (Hu et al., 2013). However, due to the high thermal stability of 3-MCPD, this technique gives poor repeatability in addition to their relatively time-consuming, their cost and their complexity with sophisticated instruments and the need of qualified personnel. Another technique which has given excellent result is electrochemical technique (Sun et al., 2014a). In order to overcome these problems, molecular imprint-based (MIP) sensor was investigated as quantitative tool for the monitoring of 3-MCPD in food products (Fang et al., 2019). Similar work has been done using electrochemical sensor based on a polyaminothiophenol modified molecularly imprinted film (Sun et al., 2014b). However, the complex extract of some food products are still interfered in the detection of 3-MCPD, making the analysis of chloropropanols isomers at the low level in foodstuffs difficult.

Furthermore, the use of nanostructures is seen as a good alternative for the detection of numerous compounds for food security (He et al., 2008; Bobrinetskiy and Knezevic, 2018). The main mechanisms and designs of nanostructured detection systems are variable and aim to the rapid and accurate detection of pathogens or chemical agents at ultra low level (Han et al., 2019).

As 3-MCPD can easily react with amine functional group of amino acid by substitution of chlorine atom of 3-MCPD in alkaline solution, the nanostructures have been designed to make the amine functional group available on AgNPs for 3-MCPD using cysteine (Martin et al., 2021a, 2021b). In this current work, a bimodal UV-Vis/electrochemical detection technique was employed to estimate the level of 3-MCPD in commonly-consumed food in Ivorian cuisine. The contents obtained by the colorimetry/UV-Vis and electrochemical methods are between not detected to 1612 μg/kg, and 97 μg/kg to 1089 μg/kg, respectively.

MATERIALS AND METHODS

Reagents and solvents

The following chemicals were used without further purification. Silver nitrate (AgNO₃, 99%), and sodium hydroxide (NaOH, 98%) were purchased from Sigma-Aldrich, and L-cysteine (C$_6$H$_7$NO$_2$S, 98%) from Merck. N-Hexane (C$_6$H$_{14}$, 99%), and acetone (C$_{4}$H$_{8}$O, 99.5%) were purchased from Pancreas. The Cys-AgNPs were synthesized according to the previously reported procedure with slight modification (Khan et al., 2012). Ultra-pure water obtained from deionized (DI) water system with a resistivity of 18.25 MΩ·cm was used throughout all the experiments.

Apparatus and Instrumentations

The UV-Vis spectra were recorded using Ocean Optics FLAME spectrometer (USA). All Electrochemical measurements were performed with a MiniEC2 Electrochemical workstation, homemade equipment provided by the Department of Information Science, East China University of Science and Technology (MiniEC2 Instruments, Shanghai, China). A conventional three-electrode system consisting of modified gold electrode (Cys-AgNPs/Au) as working electrode (Φ=1 mm), carbon leg wire as a counter electrode and a saturated calomel electrode (SCE) as reference electrode was employed. All experiments were performed at ambient temperature (around 28°C). Cys-AgNPs/Au electrode was fabricated according to the previously reported procedures (Khan et al., 2012; Martin et al., 2021a).

Sampling

A total of 297 samples namely liquid foods (palm oil and frying oil from tuna fish), fried foods, smoked foods, and roasted foods was purchased from various wholesale markets from Abobo, Adjame and Yopougon, the most popular municipalities of Abidjan (Côte d’Ivoire). Sampling was carried out considering previous data reported in the literature regarding the occurrence of 3-MCPD. Most of the products were obtained in three sectors for each of the municipalities and
in each from at least 3 different sellers. Samples were analyzed after proper homogenization, and analysis was performed in triplicate.

**Determination of 3-MCPD**

**Extraction of 3-MCPD in foodstuff**

To 5 g of sample placed in a 50 mL centrifuge tube, 30 mL of a hexane/acetone mixture (1:1, v/v) is added. The mixture is homogenized for 10 min with a vortex and then filtered with Buchner. The solid residue is washed twice with 10 mL of the same hexane/acetone mixture, and the filtrate is transferred to a separating funnel containing 10 mL of water. The lower aqueous layer is separated from the organic layer. This organic layer is re-extracted with another 10 mL portion of water. The two combined extracts are evaporated to dryness in a 100 mL distilling flask under vacuum at 55°C. One milliliter of the residue is used for colorimetric analysis (the extract can undergo dilution before UV-Vis measurement if the color change occurs without heating during its colorimetric analysis). This effect means that the concentration of 3-MCPD in this extract may be higher than the maximum of the calibration range considered in this work. It is important to note that in this extraction process, the free 3-MCPD is in the aqueous phase while its esters are found in the organic phase (Divinova et al., 2004; Karl et al., 2016).

**UV Vis detection of 3-MCPD**

To 1 mL of extracted sample, 50 μL of the prepared Cys-AgNPs solution is added, followed by 2 min vortex homogenization. Afterward, the mixture is bath-heated at 100°C for 5 min only to improve the reaction speed. The reaction leads to a change of color that can be colorimetrically detected. The concentration of 3-MCPD (C$_{3\text{-MCPD}}$) in each sample used, were investigated using the Equation (1).

\[
C_{3\text{-MCPD}} = \frac{(C \times f \times V)}{W}
\]  

where C is the found concentration from the calibration obtained in our previous study (Martin et al., 2021b), f is diluting factor, V is undiluted solution (extract of sample) and W is the weight of the sample analyzed.

**Electrochemical detection of 3-MCPD**

The estimation of 3-MCPD in the food samples was also carried out using the DPV technique by mixing 0.5 mL of the sample obtained after extraction from food products namely palm oil, frying oil from tuna fish, fried foods, smoked foods, and roasted foods in 50 mL aqueous solution of 4 M NaOH in the electrochemical cell. All DPV experiments were done between -0.5 V and 1.4 V; at scan rate 100 mV/s, pulse time 0.05 s, sample time 0.1 s, stand time 2 s, staircase potential 7 mV and pulse amplitude of 100 mV. The concentration of 3-MCPD ($C_{3\text{-MCPD}}$) in each sample used, was also investigated using Equation (1).

**RESULTS**

**Detection by UV-vis method**

The content of 3-MCPD in eleven (11) foods chosen in three municipalities of Abidjan was studied (Table 1). The extraction procedure is that described in the experimental section. This procedure is used to access the content of free 3-MCPD in the aqueous phase using Equation (1) (Divinova et al., 2004; Karl et al., 2016). As shown in Table 1, the content of 3-MCPD in these samples was found to be between not detected (below the limit of detection) and 1612 µg/kg. 3-MCPD is undetectable in millet fritters and roasted banana (18% in the studied foods). However, the contaminant was found in quantifiable amounts in smoked mackerel (233-474 µg/kg), fried tuna (403-675 µg/kg), tuna frying oil (228-1451 µg/kg), palm oil (310-1773 µg/kg), fried ripe banana (161–806 µg/kg), potato fried (156-353 µg/kg), roasted peanuts (483-804 µg/kg), banana chips (241–483 µg/kg) and pork barbecues (241–483 µg/kg).

**Detection using the electrochemical method**

The electrochemical nanosensor developed in our previous method (Martin et al., 2021a) was used to assess the content of 3-MCPD in the same 11 food matrices studied in the previous section. The results of this
investigation are summarized in Table 2. Ten (10) of these food products (90%) contain the studied contaminant, detectable at levels between 19 and 1280 µg/kg. Among these food products, except millet fritters which only contain 3-MCPD with a relatively low content (19-40 µg/kg), the other food products such as smoked mackerel (172-469 µg/kg), fried tuna (396-624 µg/kg), tuna frying oil (344-739 µg/kg), palm oil (329-1280 µg/kg), fried ripe banana (253-636 µg/kg), potato fried (160-382 µg/kg), roasted peanuts (487-787 µg/kg), banana chips (294-478 µg/kg) and pork barbecues (283-450 µg/kg) have been found to contain a significant amount of 3-MCPD.

Comparative study of the two designed methods

The content of 3-MCPD in fried, roasted, grilled, palm oil, and deep-fried foods obtained from the three municipalities using the two proposed methods were compared. As can be seen in Figure 1, the two methods exhibit almost the same order of 3-MCPD level in food matrices regardless of the sample. Both methods are therefore reliable and can be used for 3-MCPD monitoring. Moreover, although the sensitivity of the electrochemical method is lower compared to that of the UV-Vis method, this latter is easy to implement and has a short design procedure. The two methods combine high performance, linearity, reliability, and precision.

Table 1: Levels of 3-MCPD obtained from UV-Vis method in food products.

| Food products          | Sample number | Abobo Level of 3-MCPD (µg/kg) | Adjame Level of 3-MCPD (µg/kg) | Yopougon Level of 3-MCPD (µg/kg) |
|------------------------|---------------|--------------------------------|--------------------------------|----------------------------------|
|                        |               | min–max*                      | min–max*                       | min–max*                        |
| Smoked Mackerel (SM)   | 27            | 233–474                       | 313–394                        | 260–371                         |
| Fried tuna (FT)        | 27            | 403–675                       | 403–564                        | 458–656                         |
| Tuna frying oil (TFO)  | 27            | 228–1451                      | 403–645                        | 337–720                         |
| Palm oil (PO)          | 27            | 310–1773                      | 837–976                        | 887–1612                        |
| Fried ripe banana (AF) | 27            | 161–725                       | 219–806                        | 532–684                         |
| Potato fried (PTF)     | 27            | 156–229                       | 156–322                        | 282–353                         |
| Roasted peanut (RP)    | 27            | 645–804                       | 483–725                        | 483–564                         |
| Banana chips (BC)      | 27            | 241–483                       | 310–387                        | 403–465                         |
| Pork barbecue (PB)     | 27            | 241–403                       | 403–483                        | 241–403                         |
| Millet fritters (MF)   | 27            | ND                            | ND                             | ND                              |
| Roasted banana (RB)    | 27            | ND                            | ND                             | ND                              |

Min–max: minimum–maximum.
Table 2: Content of 3-MCPD obtained from the electrochemical method in food products.

| Food Product            | Sample number | City          |
|-------------------------|---------------|---------------|
|                         |               | Abobo min–max¹ | Adjame min–max¹ | Yopougon min–max¹ |
| Smoked mackerel (SM)    | 27            | 223-393       | 172-469         | 174-439          |
| Fried tuna (FT)         | 27            | 399-624       | 396-516         | 439-618          |
| Tuna frying oil (TFO)   | 27            | 685-688       | 396-679         | 344-739          |
| Palm oil (PO)           | 27            | 329-1239      | 842-941         | 895-1280         |
| Fried ripe banana (FRAB)| 27            | 253-636       | 397-458         | 534 580          |
| Potato fries (PF)       | 27            | 160-239       | 184-318         | 163 382          |
| Roasted peanut (RP)     | 27            | 717-787       | 487-714         | 510-633          |
| Banana chips (BC)       | 27            | 294-464       | 322-394         | 416-478          |
| Pork barbecue (PB)      | 27            | 289-396       | 370-450         | 283-420          |
| Millet fritters (MF)    | 27            | 23-40         | 22-26           | 19-28            |
| Roasted banana (RB)     | 27            | ND            | ND              | ND               |

¹ Min–max: minimum–maximum.

Figure 1: Comparative diagram of the average content of 3-MCPD in food products from the electrochemical and UV-Vis method.
SM (Smoked Mackerel), FT (Fried tuna), TFO (Tuna frying oil), PO (Palm oil), FRAB (Fried ripe banana), PF (Potato fries), RP (Roasted peanut), BC (Banana chips), PB (Pork barbecue), MF (Millet fritters), RB (Roasted banana).
DISCUSSION
Detection by UV-vis method
This result in Table 1 reveals the low content or the absence of 3-MCPD in these latter, and could be explained by the fact that in their production process, these two foods do not require an addition of salt. This result is similar to those of the literature (MacMahon et al., 2013; EFSA, 2016; Karl et al., 2016). Indeed, according to the literature, the 3-MCPD content varies from 26 to 39 µg/kg in these types of foods and may reflect the non-detection of 3-MCPD by the implemented method because our limit of detection is 84 µg/kg, which is significantly higher than the levels of 3-MCPD found in these products. However, the observed high values of 3-MCPD in the others can be explained by the fact that 3-MCPD comes mainly from the reaction of residual lipids and phospholipids present in the raw material and sodium chloride (naturally occurring or added) during food preparation processes exposed to high temperatures or stored for a long time period (Vicente et al., 2015). Also, the use of wood to smoke foods in the case of fish, can generate a high content of 3-MCPD (Chai et al., 2016).

Detection using the electrochemical method
The values of Table 2 are well above the acceptable level in foodstuffs set by European Commission (Stadler and Lineback, 2008; EC, 2010). In general, food products such as salted fish and pork have been shown to contain 3-MCPD after being steamed (Chung et al., 2008). From these above results, a refined oil diet, soups, and snacks can be considered as a potential source of exposure to free 3-MCPD. Moreover, it should be noted that this method was not able to detect 3-MCPD in roasted bananas. This is believed to be due to the absence of chloride ions from the food matrix itself or the addition of salt during its processing, as observed in the previous technique.

Comparative study of the two designed methods
The content of 3-MCPD in fried, roasted, grilled, palm oil, and deep-fried foods obtained from the three municipalities using the two proposed methods were compared. As can be seen in Figure 1, the two methods exhibit almost the same order of 3-MCPD level in food matrices regardless of the sample. Both methods are therefore reliable and can be used for 3-MCPD monitoring. Moreover, although the sensitivity of the electrochemical method is lower compared to that of the UV-Vis method, this latter is easy to implement and has a short design procedure. The two methods combine high performance, linearity, reliability, and precision.

Conclusion
The comparison of the performance of the two methods in the detection and quantification of 3-MCPD in selected samples, suggests that they can be used for the detection and quantification of 3-MCPD in food products from domestic or industrial processing. In addition, these methods can allow the monitoring of the content of 3-MCPD in food matrices with short time and simple sample preparation, making this work important to ensure food safety and quality for countries where these food products are mainly present in culinary menus without any controls and where it is necessary.

COMPETING INTERESTS
The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS
MAA has carried out the experiments and written the first draft of the manuscript. EKF, GY and CK have suggested the project about this work; applied for funding, followed the execution of this work, and deeply checked the manuscript writing process.
IBIW, PBBN, and KKKS have collected the samples and prepared the method of extraction. TA has supervised the electrochemical experiments.

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