Recent studies of synthetic antibody-based 3-MCPD determination technology

Penelitian terkini dari teknologi deteksi 3-MCPD berbasis antibodi sintesis

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Abstract

3-kloro-1,2-propanadiol (3-MCPD) dikelaskan oleh International Agency for Research on Cancer sebagai bahan bersifat karbon dan menjadi salah satu permintaan dari Uni Eropa yang menyarankan tingkat maksimum konsentrasi dari 3-MCPD dalam minyak sawit hingga 2,5 ppm. Meskipun metode GCMS dan HPLC-FLD yang dilaporkan menunjukkan sensitivitas dan seletifitas yang tinggi pada pengukuran 3-MCPD, semua metode tersebut membutuhkan bahan kimia yang banyak dan proses pengerjaan dengan waktu yang lama untuk preparasi dan analisis sampel. Molecularly Imprinted Polymer (MIP) atau antibodi sintetik bisa digunakan untuk mengenali 3-MCPD. MIP lebih stabil dalam kondisi suhu dan pH yang ekstrim. Artikel ini akan membahas tentang pemanfaatan MIP pada ekstraksi sampel dan analisis sampel untuk mendeteksi 3-MCPD. MIP disintesls melalui polimerisasi monomer monomer yang bergugus fungsi di sekitar 3-MCPD sebagai targetnya. Kemudian, 3-MCPD diekstrak dari MIP dengan meninggalkan sisi aktif. Oleh karena itu, sisi-sisi aktif inilah yang dapat mengikat kembali 3-MCPD baik dengan ikatan kovalen maupun non kovalen. Simulasi komputer dan eksperimen dapat menginvestigasi komposisi dari MIP. MIP dapat diproduksi menjadi kolom ekstraksi berbasis MIP (MIPSPE) dan sensor berbasis MIP. Kedua produk tersebut menunjukkan parameter analitik yang signifikan, yaitu nilai recovery lebih dari 90% dan limit deteksi kurang dari 2,5 ppm. Berdasarkan hasil kajian ini, penggunaan MIP dapat fleksibel, digunakan baik untuk ekstraksi maupun analisis sampel dalam penentuan 3-MCPD. Teknologi berbasis MIP ini akan menjadi instrument yang prospектив untuk mendeteksi 3-MCPD. Produksi MIP dalam skala industri akan menjadi sebuah tantangan dalam memonitor tingkat konsentrasi 3-MCPD dalam produk minyak sawit.

[Keywords: 3-MCPD, molecularly imprinted polymer (MIP), kolom ekstraksi berbasis MIP, sensor berbasis MIP]

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Introduction

3-chloro-1,2-propanediol (3-MCPD) is one of the critical substances for palm oil exporters, particularly in Indonesia. This material will be one of the palm oil requirements for export trade proposed by the European Commission (Suwastoyo, 2020). At this moment, the valid regulation for 3-MCPD is only focussing on hydrolyzed vegetable protein and soy sauce with a maximum level of 0.02 ppm (EU, 2018). Surprisingly, the new regulation for 3-MCPD conferred in palm oil up to 2.5 ppm has recently been proposed by the European Commission. Although this regulation is still being discussed, it is necessary to develop innovative technology for 3-MCPD control.

The incidence of 3-MCPD was first reported in 1984 by Cerbulis and friends (Cerbulis et al., 1984). Fatty acid ester and bound 3-MCPD were found in goat’s milk. Free and bound 3-MCPDs have also been identified and reported by Zelinková et al. (2006) in several edible oils such as virgin seed oils, virgin olive oils, virgin germ oils, refined seed oils, and refined olive oils from several European countries such as France, Italy, Germany, Spain, Greece, the Czech Republic, and Hungary. Some researchers in Indonesia, Lanovia and colleagues (Lanovia et al., 2014) reported that 3-MCPD was present in palm oil with a 14-35 ppm concentration range. Concerning the above, it is said that the presence of 3-MCPD or derivatized 3-MCPD in our foodstuffs should be more attentive.

The International Agency for Research on Cancer (IARC) reported the carcinogenicity of 3-MCPD in their monograph book (IARC, 2013), although no data were found in human cases. Several methods, such as oral and subcutaneous administration, and dermal application, have investigated the incidence of tumors in mice and rats (Van Duuren et al., 1974; Weisburger et al., 1981; Cho et al., 2008; Jeong et al., 2010), showing damage to multiple organs, such as liver and kidney. From these data, it is believed that to investigate the 3-MCPD residue in our food, the technique for controlling 3-MCPD is essential; therefore, organ damage would be immediately anticipated.

This paper aims to discuss the relevant information on the latest technology based on molecularly imprinted polymer, particularly its performance and application in actual samples. General information on 3-MCPD has been provided, including physical and chemical properties. The authors also described earlier technologies from the past, such as Gas Chromatography-Mass Spectroscopy (GC-MS) and High-Performance Liquid Chromatography Fluorescence Detector (HPLC-FLD). Molecularly Imprinted Polymer (MIP) was mentioned as a synthetic antibody, including composition, production, and for solid-phase extraction (SPE) and sensor application. In the end, the future development of MIP-based technology has been identified from previously reported developments.

3-MCPD: physical and chemical characteristics

The 3-MCPD has more than ten names and 3-monochloro-1,2-propanediol is the popular one (IARC, 2013). 3-MCPD has a molecular weight of 110.54 g mol⁻¹ and is soluble in water, alcohol, diethyl ether, and acetone. From the chemical formula, 3-MCPD contains one chloride and two hydroxy groups (Figure 1). It is called the unbound or free 3-MCPD. Other forms of 3-MCPD are bound 3-MCPD containing the ester chain and could be hydrolyzed to be a free form (Barocelli et al., 2011; Abraham et al., 2013).

The formation of 3-MCPD can be triggered in palm oil during fat frying by sodium chloride (NaCl), water content, heating temperature, and heating time (Zhou et al., 2014). From these results, careful processing of palm oil could be recommended. There are many cases in which 3-MCPD may be formed during degumming (Zulkurnain et al., 2012; Matthäus & Pudel, 2013), neutralisation (Matthäus & Pudel, 2013), bleaching (Razak et al., 2012; Zulkurnain et al., 2012) and deodorisation (Franke et al., 2009; Hrncirik & van Duijn, 2011). Due to these issues, the 3-MCPD is easily left in food.

![Chemical structures of free 3-MCPD and bound 3-MCPD](image)

Figure 1. Chemical structures of free 3-MCPD and bound 3-MCPD

Gambar 1. Struktur kimia 3-MCPD bebas dan 3-MCPD terikat

Source: modified from Andres and colleagues (2013)
Sumber: dimodifikasi dari Andres et al. (2013)
The current technologies for 3-MCPD detection

Most of the techniques used to detect 3-MCPD are based on chromatography methods. One of them is GC-MS. This method is probably not easily accessible due to lengthy procedures for sample extraction and analysis, even though the European Commission recommended the GC-MS method (EU, 2014). Miyazaki and colleagues (Miyazaki et al., 2012) tried to analyze 3-MCPD of spiked palm oil. The oil sample was hydrolyzed and derived before going to GC-MS. It is claimed that 3-MCPD was read as 3-MCPD derivatization, i.e., dipalmitic 3-MCPD dioleic, 3-MCPD, 3-MCPD dilinoleic, 3-MCPD 1-palmitate, and 3-MCPD 1-oleate. Results from a recovery test of 93-106% were quite good. They also applied this method to other samples with a different matrix, such as rapeseed oil, safflower oil, olive oil, soya bean oil, corn oil, rice bran oil, sesame oil, and sunflower oil, with a recovery test of between 89 and 108%.

The following method is HPLC-FLD, reported by Hu et al. (2013), testing 3-MCPD in vegetable oils, including corn oil, rice bran oil, and soya oil. The procedure is different from the one before. The 3-MCPD was cleaved by chloroacetaldehyde and derivatized by adenine, finally producing εAde (1-N⁶-etheno adenine). FLD then analyzed this final product through Fluorescence Derivation. The results of this experiment were terrific since the recovery test was approximately 93 – 97%, and the detection limit was 0.36 ppb. The last example is from Lanovia et al. (2014), measured free 3-MCPD and total 3-MCPD in palm oil measured by GC-MS. The instrument was modified by the Weißhaar method (Weißhaar, 2008). The experiments claimed that the results were outstanding for several reasons, such as concentration range: 0.008-0.377 ppm, detection limit: 0.06 ppm and recovery test: 96-113%.

Thus far, the current technologies (GCMS and HPLC FLD) for 3-MCPD detection show powerful results even though they are still laborious and time-consuming. The methods use many chemicals and are not easy for people to operate the instrument. Besides, there is no guarantee that 3-MCPD will be fully converted to its derivatization product event though the methods enable to measure the derivatisation of 3-MCPD. As a result, MIP-based technology could minimize the problem of those technologies to identify better 3-MCPDs than previously reported (Table 1).

| Methods for 3-MCPD analysis | Advantages | Disadvantages |
|----------------------------|------------|---------------|
| Metode analisis 3-MCPD    | Kelebihan  | Kekurangan    |
| GC-MS                     | - High sensitivity and selectivity | - Long protocol for sample preparation consuming the plenty of chemicals |
|                          | - Enable to detect multianalyte with different analogs | - Long procedure for sample analysis consuming the plenty of chemicals; therefore, a trained person is needed |
|                          |           | - The maintenance fee is expensive |
|                          |           | - The price for sample analysis is expensive |
| HPLC-FLD                  | - High sensitivity and selectivity | - Long protocol for sample preparation consuming the plenty of chemicals |
|                          | - Enable to detect multianalyte with different analogs | - Long procedure for sample analysis consuming the plenty of chemicals; therefore, a trained person is needed |
|                          |           | - The maintenance fee is expensive |
|                          |           | - The price for sample analysis is expensive |
| MIP-based technologies    | - Short protocol for sample preparation consuming less chemicals | - Cannot detect multianalyte with different analogs because the MIP is produced for only one single target. |
|                          | - Short procedure for sample analysis and no need to hire a trained person | |
|                          | - Low cost for maintenance fee | |
|                          | - The price for sample analysis is cheap | |
|                          | - High sensitivity and selectivity | |
Molecularly Imprinted Polymer (MIP)-based technologies

MIP composition for 3-MCPD

In general, several chemicals such as functional monomers (FMs), crosslinkers (CL), initiators (I), and solvents (S) are needed to produce MIP. FMs are critical materials because these chemicals should have a proper interaction directly with 3-MCPD; therefore, the MIP has an active cavity recognizing 3-MCDP correctly. Published articles (Leung et al., 2003; Li et al., 2014; Sun et al., 2014; Fang et al., 2019; Yaman et al., 2020) show that FMs for 3-MCPD had different materials among experiments (Table 2). It is indicated that FM materials can be flexible as long as these chemicals can react with 3-MCPD. Similarly, the solvents used in each MIP production are different depending on the solubility of FMs and 3-MCPDs. These reagents are also crucial to produce MIP on the polymerization step. CL is probably a similar reagent for different MIP production methods and different MIP applications, unlike FMs and solvents. Similarly, the use of an initiator occurred, as shown in Table 2. For instance, ethylene glycol dimethacrylate and Azodi isobutyro nitrile are popular materials as CL and Initiator, respectively. They can be commonly used for bulk polymerization and both sample preparation and analysis purposes. Besides, not all of the MIP production needs CL and Initiator. For example, Sun et al. (2014) did not use CL, while Yaman et al. (2020) did not use I to generate MIP. The main reason is that they used the method of electropolymerization for the construction of MIP. Therefore, the composition of the MIP is very versatile, and it would be an opportunity for the researcher to explore the new composition of the MIP occasionally.

Table 2. The composition, method, and application for 3-MCPD imprinted polymer

| MIP composition/ Komposisi MIP | Method Methode | Application Aplikasi | Reference Referensi |
|--------------------------------|----------------|----------------------|---------------------|
| p-amino thiophenol - Tetraammonium perchlorate | Electropolymerization | Sample analysis | (Sun et al., 2014) |
| 4-vinyl phenyl boronic acid Ethylene glycol dimethacrylate Azodi isobutyro nitrile | Chloroform Methanol: water Ethanol | Bulk polymerization Sample extraction Sample analysis | (Leung et al., 2003) (Li et al., 2014) (Fang et al., 2019) |
| Methacrylic acid | | | |
| Pyrrole Graphene oxide | Methanol | Electropolymerization Sample analysis | (Yaman et al., 2020) |

Notes:
FM= Functional Monomer, CL= Cross Linker, I/ES= Initiator/Electrolyte Solvent, S= solvent

Keterangan:
FM= monomer fungsional, CL= bahan pengikat, I/ES= inisiatorelektrolit, S= pelarut
There are many reported MIP production methods, such as conventional polymerization (e.g., bulk polymerization, suspension polymerization) (De Smet et al., 2009), supercritical fluid technology (Scholsky, 1993), solid-phase synthesis (MIP in nanoscale, called nanoMIP) (Munawar et al., 2020a) and electropolymerisation (Munawar et al., 2020b). Unfortunately, according to the published article on the development of MIP for 3-MCPD, only two methods are described here, bulk polymerization (Li et al., 2014) and electropolymerisation (Sun et al., 2014; Yaman et al., 2020).

**MIP based SPE (MIPSPE) for 3-MCPD**

The preparation of MIPSPE is more straightforward than reported SPE, and there is no need to spend many chemicals. Figure 2 showed how to form the MIP for 3-MCPD modified from Li et al. (2014). The silica gel was activated by sodium hydroxide and silanized by APTES, obtaining the modified silica gel. This silica gel was then used for copolymerization of MAA (FM) and EGDMA (CL) in the presence of 3-MCPD. Copolymerization occurred due to the presence of AIBN, resulting in a non-covalent MAA and 3-MCPD complex. In this case, 3-MCPD was predicted to form hydrogen bonding with MAA (Figure 2(a)). MCPD was removed by methanol: water (9:1, v/v) to obtain pure MIP. Li also provides computational simulation data to prove the interaction between 3-MCPD and MAA (Figure 2b). They claim that the best ratio is 1:2 for 3-MCPD and MAA, respectively, for MIP production. In addition, the morphology of the produced MIP for 3-MCPD was characterized using SEM. FTIR also observed the identification of the structure of MIP. The MIP profile could, therefore, be more comprehensive and reliable.

Figure 2

(a) Illustration of MIP production: (1) activation and silanization of silica gels (2) copolymerization of MIP on the surface of modified silica gels (3) removal of 3-MCPD for getting active cavities of MIP. (b) Associated complexes formed between 3-MCPD and MAA; the dotted lines indicate the hydrogen bonds using HyperChemV8.0.1.

Gambar 2

(a) Ilustrasi dari produk MIP: (1) aktivasi dan silanisasi dari gel silika (2) kopolimerisasi MIP pada permukaan gel silika yang sudah dimodifikasi (3) pelepasan 3-MCPD untuk mendapatkan sisi aktif dari MIP. (b) Kompleks senyawa antara 3-MCPD dan MAA; garis titik titik mengindikasikan ikatan hydrogen menggunakan HyperChemV8.0.1.

Source: modified from Li et al. (2014)

Sumber: dimodifikasi dari Li et al. (2014)
Recent studies of synthetic antibody-based 3-MCPD ..............................................(Munawar et al.)

**MIP-based sensor for 3-MCPD**

The next application, MIP, may also be used for sample analysis. Yaman et al. (2020) indicated that the MIP might be manufactured as a 3-MCPD sensor. Based on its outcome, this sensor is assumed to be quite good. The sensor performance was worked on the concentration range at 5-500 nM and the detection limit was 1.82 nM. The interesting point is how they manufactured the sensor using MIP (Figure 3), even though they still used old-fashioned electrode systems, including pencil electrode graphite (working electrode), Platinum wire (counter electrode), and Ag / AgCl/3 M KCl (reference electrode), instead of the screen-printed electrode used recently by so many sensor developers.

Manufacturing MIP-based sensor uses electropolymerization that is simpler than bulk polymerization. Here, Yaman and colleagues (Yaman et al., 2020) used a pencil graphite electrode (PGE) as a working electrode where the MIP attachment is located. The protocol is a one-time step, but they instantly get both the MIP and the MIP-based sensor. For this reason, electropolymerization is not complicated and is also more economical because few chemicals are used. Figure 3 shows the production of MIP and

| Type of sample | Type of SPE | Reagent | Recovery (%) | LoD Limit deteksi (ppm) | Reference |
|----------------|-------------|---------|--------------|------------------------|-----------|
| Soya sauce     | MIP SPE     | acetonitrile: water | 93           | 0.002                  | (Li et al., 2014) |
| Cookies and Margarine | diatomaceous earth extraction | ethyl ether: hexane | 99 - 108 | NA                    | (Becalski et al., 2015) |
| Soy sauce, dehydrated soup, toasted bread, vegetable oils, salami, sausage, and cheese | ExtrelutÔ NT20 cartridges | ethyl acetate | 92 – 100 | NA                    | (Retho & Blanchard, 2005) |
| Vegetable oil | C18 powder-packed syringe | acetonitrile: 2-propanol | 94 – 108 | 0.0001–0.02 | (C. Li, Nie, Zhou, & Xie, 2015) |
| Infant Formula Milk Powder | alkaline diatomite SPE column | acetic ether: diethyl ether | 98 – 111 | 30 | (Wang et al., 2016) |
| Corn oil       | DVB/CAR/PDMS packed column | NA | 93 | 0.004 – 0.005 | (Xu, Jin, Yang, Rao, & Chen, 2020) |
| Extra virgin olive | (Si-SAX and PSA)-packed SPE tube and (Z-Sep+ and PSA)-packed SPE | diethyl ether: hexane and ethyl acetate: acetonitrile | 71 – 123 | 10 – 20 | (Custodio-Mendoza et al., 2018) |

Figure 3. The illustration of MIP-based sensor for 3-MCPD using pencil graphite electrode (PGE)

Gambar 3. Ilustrasi dari sensor berbasis MIP untuk 3-MCPD menggunakan elektroda grafit pensil

Source: modified from Yaman et al. (2020)

Sumber: dimodifikasi dari Yaman et al. (2020)
sensor. Step one, the graphene oxide (GO) was immobilized on the surface of the PGE. The pyrrole and 3-MCPD were then electropolymerized by Cyclic Voltammetry in optimum condition (Britton Robinson buffer, pH 4), potential range 0.0+1.2 V (vs. Ag/AgCl), scan rate 50 mV/s, five cycles) on the GO-modified PGE (GO / PGE) surface. The last step was to wash the PGE with methanol: acetic acid (9:1, v / v) to remove 3-MCPD from the system and leave the active MIP-based sensor (MIP(oPPy)/GO / PGE) cavities. The sensor is finally used for 3-MCPD detection on actual samples, soya sauces from the local market, using electrochemical impedance spectroscopy (EIS) with the optimum setting (AC potential +0.20 V, frequency range 0.1 Hz-100 kHz, amplitude ten mV, ferri/ferrocyanide redox system). However, the data is likely to be validated to determine the recovery percentage in further study; therefore, the sensor is more reliable.

Sun and Colleagues (Sun et al., 2014) also applied MIP on sensor technology. However, the manufacturing process of the sensor is more complicated than the previous explanation, even though both used the same conventional electrode system. Herein, they used a modified carbon glass electrode (GCE) as a working electrode, a platinum wire as a counter electrode, and a saturated carbon electrode (SCE) as a reference electrode. Therefore, it is believed that there is an opportunity to develop a 3-MCPD sensor with a screen-printed electrode because it would be more portable and moveable. The sensor was manufactured in several stages (Figure 4) because the GCE should be modified by tetrachloroaurate (III) acid (HAuCl4) to form Au nanoparticles (AuNPs) deposit. The surface of the AuNP modified GCE (AuNPs / GCE) was then self-assembled by p-amino thiophenol (p-ATP) through interaction between the gold and thiol groups (-SH) of the p-ATP to form an Au−S bond. The 3-MCPD was continuously immobilized on that electrode, employing hydrogen bonding between the amino groups of p-ATP and the hydroxyls of 3-MCPD. Finally, the MIP was electropolymerized by Cyclic Voltammetry with optimum condition (10 cycles, potential range −0.3–+1.2 V, scan rate 50 mV/s).

The outstanding results have been reported, although the protocol for manufacturing the MIP-based sensor has been complicated. The produced sensor was tested for its 3-MCPD solution with a concentration range of 1.0 × 10^{-17} to 1.0 × 10^{-13} M, and the sensor is quite sensitive because the detection limit is lower than 1 nM. Soya sauce was also used for the recovery test, resulting in a recovery percentage of more than 90%. There is, however, no data on the consistency of the sensor during the time of storage and no explanation of the sensor's reproducibility.

![Figure 4](image_url)
Recent studies of synthetic antibody-based 3-MCPD ………………………………..(Munawar et al.)

The selectivity of MIP-based technologies

MIP selectivity was reported from Li and Colleagues (2014), showing results from MIP-based SPE. Figures 5a and 5b illustrated the chromatograms of mixture solution including 3-MCPD, 2-MPCD, and 1,3-DCP, without and with MIP-based SPE pre-treatment. The performance of MIP was very outstanding in distinguishing 3-MCPD from Li’s work with other targets. In addition, the selectivity of MIP was observed by Leung et al. (2003), producing an electrochemical sensor based on MIP. The 3-MCPD sensor may differ from other analog diols such as 1,2-propanediol and 1,3-propanediol, and 1,3-DCP (Figure 5c). It appears that the sensor was unable to recognize the other targets as the same as those responses from the control material. Similar results have been shown in the following reports (Sun et al., 2014; Fang et al., 2019; Yaman et al., 2020).

More analog diols (e.g., glycerol, 2,3-DCP, ethylene glycol, 2-MCPD, L-glutamic acid, L-tryptophan and L-phenylalanine) were used to demonstrate the selectivity of MIP in the flatform sensor, and the performance of MIP did not significantly recognize those targets. Unfortunately, no observation has been reported about the selectivity of MIP on free and bound 3-MCPDs. It would be an excellent opportunity for future researchers to investigate this challenge.

Conclusion

Molecular Imprinting Polymer (MIP) technology is an alternative method for controlling 3-MCPD with many benefits. The few chemicals used are advantageous to MIP. For this reason, the use of MIP has so far been safer than other methods. The application of MIP is also applicable to the extraction and analysis of samples. For some analysts/users, the extraction step is laborious, not only chemicals used but also the time and effort needed. The MIP-SPE would be more effective. Therefore, the improvement of this innovative SPE should be made to achieve not only acceptable but also reliable results. The following future, probably the validation step, is an improvement to achieve better MIP-SPE quality than previously reported. Furthermore, the MIP-based sensor technology showed the robust performance of the soya sauce even though the old-fashionable electrode systems are still used. Further research, therefore, requires the use of a screen-printed electrode to make the sensor more portable. Testing on actual samples is also required in a variety of foods containing 3-MCPD or bound 3-MCPD.

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