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Original article

Determination of 2-MIB and rancid-related volatile lipid oxidation products in hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) with an automated HS-SPME-GC–MS-QTOF-arrow technique

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ABSTRACT

A headspace solid–phase microextraction (HS-SPME) method coupled with gas chromatography/mass spectrometry with quadrupole time-of-flight (GC/MS-QTOF) was developed for analysis of volatile off-odor compounds, i.e., earthy/musty (2-methylisoborneol, 2-MIB) and rancid (aldehydes and alcohols), from farmed hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*). The most efficient extraction of targeted volatiles was provided by 50 min at 70 °C with a CWR-PDMS fiber and 3 g of fish diluted to 5 mL with 1.5 g NaCl (30 % saturated NaCl). The maximum time-delay before extraction was 8 h to avoid spoilage and lipid oxidation during analysis. The final method showed good linearity, intraday repeatability of 5–9 %, interday reproducibility of 5–12 % and recoveries of 94–112 %. The implementation part proved that the developed method gave accurate quantitative results for oxidation-derived volatiles, several with high correlation to thiobarbituric acid reactive substances (TBARS). Altogether, our study provided an effective SPME-GC–MS method for the extraction and analysis of important off-odor compounds in catfish mince.

1. Introduction

2-Methylisoborneol (2-MIB) is, together with geosmin, known to be the major causes of earthy-muddy odor in fish, which is connected to large economic losses in the aquaculture industry. As an example, only 10 % addition of channel catfish surimi into crabsticks was considered resulting in a muddy odor taint [1]. Humans can sense these 2 compounds at extremely low levels; part per billion (pbb) in a fish matrix (i.e., 0.1–0.5 ng/g) and part per trillion (ppt) in a water matrix (i.e., 35–40 pg/mL) [2–4]. In fresh channel catfish (*Ictalurus punctatus*) fillets, the sensory thresholds of 2-MIB and geosmin has been reported to be 0.1–0.2 and 0.25–0.5 ng/g, respectively [1]. Our previous study found concentrations of 2-MIB and geosmin levels in a hybrid catfish matrix at 0.76 and 0.14 ng/g, respectively [5]. Another of our previous studies found that 2-MIB and geosmin levels were above its threshold in all muscle samples taken throughout the entire body of farmed hybrid catfish [6]. Moreover, our recent work revealed that not only 2-MIB and geosmin, but also (*E*)-2-nonenal and 1-octen-3-ol intensified the earthy and muddy odor in farmed hybrid catfish muscles [7]. Thus, efficient analysis methods with low limits of detection are needed for all these volatiles; not least for 2-MIB which has been less researched than geosmin.

The traditionally used solvent-free microwave mediated distillation (MD) extraction method for 2-MIB, provide a low limit of detection (LOD); 0.001 ppb [8] and 0.01 ppb [9] (Table 1). Unfortunately, this technique is labor-intense, requires large fish samples (~20 g) and has low recovery yield. SPME, on the other hand, can overcome those limitations, and has several well-known advantages, including simplicity, high sensitivity, and a relatively non-invasive nature [10]. However, Thomsen et al. [11], reported that the more complex the sample matrices were, the higher the problems with competition for fiber surface area and overestimation of volatile compounds in SPME-based analyses. Fish samples, having a large variety of proteins and lipids, can thus easily encounter such issues due to their susceptibility to e.g.,

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protein and lipid oxidation. The latter is stimulated by operations as mincing which breaks down membranes and thereby exposes polyunsaturated fatty acids (PUFA) to enzymes and non-enzymatic catalysts such as heme iron, nonheme iron, and other trace metal cations [12,13]. Protein oxidation can be generated directly by reactive oxygen and nitrogen species, or it can be caused indirectly by interactions with products of lipid oxidation or with reducing sugars or carbohydrates [14]. Since the holding time for each sample in a sample rack before extraction is not equal when applying automation in the SPME-GC–MS technique, under- or overestimations of volatile compounds in fish tissue can thus easily occur. To avoid this, the maximum holding time for fish samples in a sample rack should be established.

The SPME-Arrow is an SPME-based device invention created to improve sensitivity in volatile compound analyses by increasing the volume of the extraction phase with the goal of boosting trace levels of targeted compounds [10]. The SPME-Arrow technique was recently used to enrich trace-levels of volatile terpenes (α-pinene, limonene, linalool, and citronellol) in tilapia (*Oreochromis niloticus*), with a satisfactory result due to the enhanced sensitivity and wider range of detected compounds [20]. SPME coupled with the novel Arrow fiber to establish 2-MIB and volatile oxidation product levels in fish matrices have however not yet been studied. Based on the very low level of particularly 2-MIB and *cis*-4-heptenal in fish, and the high complexity of the fish matrix, we here hypothesized that enrichment of this volatile and volatiles derived from lipid oxidation with the Arrow fiber and analysis with automated HS-SPME-GC–MS-QTOF, would be a successful approach for quantification. Full automation of the HS-SPME-GC–MS-QTOF technique is however challenging for fish tissue due to the mentioned continuous changes of volatiles taking place during extended holding times in a sample rack. The suggested analytical combination, SPME-Arrow-GC–MS, must thus be appropriately optimized prior to its use, which was the aim of this study. A secondary aim was to apply this technique to hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) to study the release of both 2-MIB and lipid-oxidation derived volatiles during ice storage. The latter were also correlated to thiobarbituric acid reactive substances (TBARS) data.

2. Materials and methods

2.1. Chemicals and materials

Propanal, hexanal, *cis*-4-heptenal, octanal, 1-hexanol, nonanal, 1-octen-3-ol, and 2,4-heptadienal were purchased from Tokyo Chemical Industry (TCI) (Tokyo, Japan). 2-MIB, *trans*-2-heptenal, and 2-methy-3-heptanone (internal standard, ISTD) were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Alkane standard mixture of C7–C40 were purchased from Supelco, Inc. (Pennsylvania, USA). Four SPME Arrow fibers were tested, including polydimethylsiloxane (PDMS), 100 µm phase thickness, and carbon wide range/polydimethylsiloxane (CWR-PDMS), 120 µm phase thickness, obtained from CTC analytics (Switzerland), as well as divinylbenzene/polydimethylsiloxane (DVB-PDMS), 120 µm phase thickness, and divinylbenzene/carbon wide range/polydimethylsiloxane (DVB-CWR-PDMS), 120 µm phase thickness, obtained from Thermo scientific (Switzerland). The phase length of all SPME Arrow fibers was 20 mm.

2.2. Fish samples

Farmed hybrid catfish (*C. macrocephalus* × *C. gariepinus*) with an average weight of 500–600 g was purchased from Thasala market, Nakhon Si Thammarat, Thailand. Fish were immediately packed in polystyrene foam boxes filled with ice (fish:ice ratio of 1:2, *w*/w) and were brought to the laboratory within 20 min. The fish were washed, headed, eviscerated, and filleted. Then, the fillets were minced using a grinder (MK 5087 M Panasonic Food Processor, Selangor Darul Ehsan, Malaysia, which was referred to as "fish mince" which used in fish weight parameter of optimization studies. Section 2.4.1 discusses the preparation of fish samples for an ice storage study.

2.3. Optimization studies

SPME parameters including fiber types, extraction times, extraction temperatures, fish weights, and aqueous NaCl concentrations were

Table 1
Comparison of sampling and analysis methods, limit of detection (LOD), limit of quantification (LOQ) and linear range parameters documented for determining 2-MIB contents in fish tissue using an ion-based method.

Sampling and analysis method[a]	Fish species	Content (ng/g)	LOD (ng/g)	LOQ (ng/g)	Linear range (ng/g)	References
	Rainbow trout (*Oncorhynchus mykiss*) fillets	10.2	0.5	20	20–60	[4]
HS-SPME-GC-MS	**European whitefish** (*Coregonus lavaretus*)	24	0.5	20	20–60	[15]
	Channel catfish *(Ictalurus punctatus)*		5.77	10	10 - 50	[16]
	Nile tilapia (*Oreochromis niloticus*)					
	mince	ND				
	dorsal	ND				
	ventral	ND	5	25	25 – 400	[17]
	Broadhead catfish (*Clarias macrocephalus)*					
	mince	0.8				
	dorsal	0.9				
	ventral	1.4	5	25	25 – 400	[17]
	Grey mullet *(Mugil cephalus)*					[18]
LLE-GC-MS	roe	63.3 – 86.2	1	5	10-100	
	Catfish *(Ictalurus punctatus)*		0.01	0.1	0.1 - 30	[9]
	Sturgeon (*Acipenser transmontanus*) fillets	0.008-0.075	0.001	-	-	[8]
	Largemouth bass (*Micropterus salmoides*) fillets	0.017-0.067	0.001	-	-	[8]
	Hybrid catfish					
	fillet mince	ND				
	skin	ND	0.01	-	-	[19]
	Channel catfish					
	frame	ND				
	head	ND				
	skin	ND				
	mince	ND				
MD-SPME-GC-MS	viscera	ND	0.01	-	-	[19]

[a] Definitions: HS-SPME-GC–MS, Headspace solid phase microextraction coupled to gas chromatography–mass spectrometry; LLE-GC–MS, Liquid-liquid extraction coupled to gas chromatography–mass spectrometry; MD-SPME-GC–MS, Microwave mediated distillation with solid phase microextraction coupled to gas chromatography–mass spectrometry; ND, not detected; "-"means not analyzed.

experimentally studied to improve the extraction efficiency of the targeted off-odor compounds. All experiments were performed in three replications. Different fish weights (2, 3, and 4 g) were placed in 20 mL headspace vials, and deionized (DI) water (3, 2, and 1 mL) was added to reach a similar volume, 5 mL, of the final sample solution. Four different extraction temperatures (40, 50, 60, and 70 °C) were tested, and four different extraction times (20, 30, 40, and 50 min). Further, the effect of NaCl addition (0, 0.5, 1, and 1.5 g per 5 g sample solution) was evaluated. The incubation and extraction procedures were carried out using a PAL RSI 120 autosampler (CTC Analytics, Zwingen, Switzerland).

2.3.1. The influence of holding time in autosampler with and without salting-out

Underestimation and/or overestimation of off-odor volatile compounds from catfish mince were evaluated before the studies of SPME-Arrow-GC–MS-QTOF. The peak areas for assayed compounds and the standard deviation (SD) were used to examine parameter optimization, and all parameters were replicated three times. Different holding times (0, 4, 8, 12, 16, and 24 h) in the autosampler were tested, without and with NaCl addition (0 and 1.5 g per 5 g sample solution).

2.3.2. GC–MS conditions

The GC–MS analysis was performed on an Agilent 7890B GC system (MA, USA) equipped with an Agilent 7250 time-of-flight mass spectrometer (MA, USA). Samples were analyzed with a capillary column DB-WAX (30 m × 0.25 mm, 0.25 μm, Agilent Technologies, CA, USA). High purity (99.999 %) helium was used as the column carrier gas at a constant flow rate of 1 mL/min. The oven temperatures were programmed: held at 45 °C for 2.5 min, increased at 10 °C min^{-1} to 80 °C, raised at 10 °C min^{-1} to 250 °C and held for 2 min. The MS spectra were recorded in the electron ionization mode (EI) at an ionization energy of 70 eV. The mass range was 35–350 *m/z*, which was scanned at 4.4 scan s^{-1}. The scan mode was used for the MS data acquisition and the extraction of ion chromatograms was used for the quantitation analysis. Identification of the volatile compounds was performed based on comparisons of their mass spectra, and retention indices (RIs) with those of pure standards (STD) under the same chromatographic conditions as was described for the samples. The RIs were calculated using a mixture of C7–C19 hydrocarbons. Furthermore, the RI's targeting compounds were compared with RI's reported in the literature (Table 2).

2.3.3. Validation parameters

2-methyl-3-heptanone was used as internal standard (ISTD). Its linear range and correlation coefficient (R^2) were determined using a matrix matched calibration method. The fish mince samples were spiked with an authentic standard mixture (2-MIB, propanal, hexanal, *cis*-4-heptenal, octanal, *trans*-2-heptenal, 1-hexanol, nonanal, 1-octen-3-ol and 2,4-heptadienal) at 1–1000 ng/g fish using a volume of 50 μL standard mixture solution. Non-spiked fish mince samples were also used, and the obtained peak areas were subtracted from the peak areas of the spiked fish samples. Then, the ratio of the authentic standard peak areas to the ISTD peak area against the concentration was calculated as matrix matched calibration curves. Limit of quantitation (LOQ) of each compound was selected from the lowest point of the curve with a good R^2 value (>0.95). Thereby, LOD of each compound was calculated from the relative standard deviation (RSD) of eight replications of spiked fish samples at the concentration of their LOQ, multiplied by two.

Intraday repeatability and interday reproducibility were calculated from the percent RSD based on three replications of spiked fish samples at the concentration of 1000 ng/g. The interday reproducibility was evaluated over three consecutive days. The accuracy was determined by examining the % recovery, which was evaluated with three replications of three different concentrations: the lowest, middle, and highest point of the curve with a satisfactory R^2 (>0.95) for each of the assayed compounds.

2.4. Ice storage study

2.4.1. Fish sample preparation

Ice storage was then performed as described by Chaijan, Benjakul, Visessanguan, and Faustman [21]. The whole fish was washed and directly placed in ice, which was distributed uniformly between the layers of fish at a fish:ice ratio of 1:2 (*w*/w). The box containing fish and ice was stored at 4–6 °C for 15 days. Every 2 days melted ice was removed and replaced with the same quantity of new ice. During the storage, fish were randomly sampled at day 0, 3, 6, 9, 12, and 15 for analyses. Upon sampling, the whole fish was immediately washed, headed, eviscerated, and filleted. To prepare the samples for analyses, they were excised into dorsal line, lateral line, and ventral plus belly flap muscles. The different muscles were then ground into a mince using a MK 5087 M Panasonic Food Grinder (Selangor Darul Ehsan, Malaysia), and the mince was stored at −20 °C until analysis, which meant at the most 3 weeks.

2.4.2. Thiobarbituric acid reactive substances (TBARS)

TBARS determinations were done following the methods by Buege and Aust [22]. The ground sample (0.5 g) was homogenized with 2.5 mL of a solution containing 0.375 % TBA (*w/v*), 15 % trichloroacetic acid (TCA) (w/v) and 0.25 M HCl. The mixture was heated in a boiling water bath (95–100 °C) for 10 min which led to development of pink color, cooled with running tap water and centrifuged at 3600 ×*g* at 25 °C for 20 min. The absorbance of the supernatant was measured at 532 nm. A standard curve was constructed using 1,1,3,3-tetramethoxypropane at concentrations ranging from 0 to 6 ppm. TBARS was expressed as mg malondialdehyde (MDA) equivalents/kg sample.

2.5. Statistical analysis

Duncan's multiple-range test was used to analyze significant differences ($p < 0.05$) among samples. Correlation analysis (Two-tailed, Pearson) between the assayed volatile compounds and TBARS was also performed. All data analysis was done by the SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussions

3.1. Study of individual method parameters

3.1.1. Arrow fiber types

Although the highest standard deviations (SD's) of almost all assayed off-odor compounds were found for the CWR-PDMS and DVB-PDMS fibers, the CWR-PDMS fiber also offered the highest response for 2-MIB, as well as for *cis*-4-heptenal, octanal, *trans*-2-heptenal, nonanal, and 2,4-heptadienal (Fig. 1a). Due to its significantly higher extraction ability of 2-MIB compared to the other fibers, the CWR-PDMS Arrow fiber was thus chosen to perform SPME analyses of the different fish samples. Using this Arrow fiber, hexanal was found to be more abundant than the other volatile lipid oxidation products compounds, and the extraction capacity of this aldehyde was found to be comparable to that obtained with the PDMS, CWR-PDMS, and DVB-PDMS fibers.

3.1.2. Fish weights

Different amounts of fish (2, 3 or 4 g made up to 5 mL with water) were tested to examine the matrix effect on extraction of off-odor compounds from hybrid catfish (Fig. 1b). According to the findings, the greatest release of hexanal, octanal, *trans*-2-heptenal, nonanal, 1-octen-3-ol, and 2, 4-heptadienal was observed when using the minimum amount of fish, i.e., 2 g. As described by Thomsen et al. (2016), when the concentration of volatile compounds in a sample increase, their affinity for a conventional SPME fiber also increases, creating competition between hexanal and other volatile compounds they assayed. Thus, competition among volatiles for the Arrow SPME fiber

Table 2
Off-odor target compounds, identification parameters, validation parameters (linear range, R^2, LOD, LOQ, intraday variation, interday variation, and recovery in farm-raised hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*).

Retention time (min)	Off-odor volatiles	Retention index		Selected ion [m/z][c]	Linear range (ng/g)	R^2	LOD (ng/g)	LOQ (ng/g)	RSD (%)[d]		Standard addition concentration (ng/g)	Recovery (%)
		Libraries[a]	Calculation[b]						Intraday variation (Repeatability)	Interday variation (Reproducibility)		
2.672	propanal	798	789	58*, 29	100–1000	0.9646	17.24	100	6	8	100	108
											500	110
											1000	107
6.237	hexanal	1083	1085	44*, 56, 82	200–1000	0.9849	151.41	200	9	8	200	112
											500	109
											1000	108
8.774	*cis*-4-heptenal	1240	1247	84*, 94	10–1000	0.9963	0.45	10	7	6	10	107
											100	95
											1000	105
9.495	octanal	1289	1295	67*, 41, 81	200–1000	0.9957	61.76	200	5	10	200	106
											500	108
											1000	108
10.047	*trans*-2-heptenal	1323	1334	83*, 69	10–1000	0.9829	8.96	10	5	6	10	104
											100	94
											1000	96
10.396	1-hexanol	1355	1359	56*, 69	10–1000	0.9927	5.15	10	8	6	10	96
											100	95
											1000	108
10.975	nonanal	1391	1400	58*, 95, 81	200–1000	0.9941	75.40	200	6	8	200	106
											500	106
											1000	109
11.699	1-octen-3-ol	1450	1454	72*, 57	10–1000	0.9925	4.49	10	8	5	10	105
											100	107
											1000	108
12.367	2,4-heptadienal	1495	1505	81*	10–1000	0.9907	5.66	10	8	8	10	95
											100	94
											1000	95
13.694	2-MIB	1592	1612	95*, 108, 135, 150	1–1000	0.9938	0.28	1	8	12	1	105
											100	94
											1000	104

[a] Retention index libraries: matching with the MS library of NIST (Version 14).
[b] Retention index calculation: calculated in relation to the retention time of n alkane (C7-C19) series.
[c] Selected ion: the asterisk represents quantitative ion.
[d] The hybrid catfish mince with authentic standard mixture at 1000 ng/g was used for calculating RSD of intraday and interday variations.

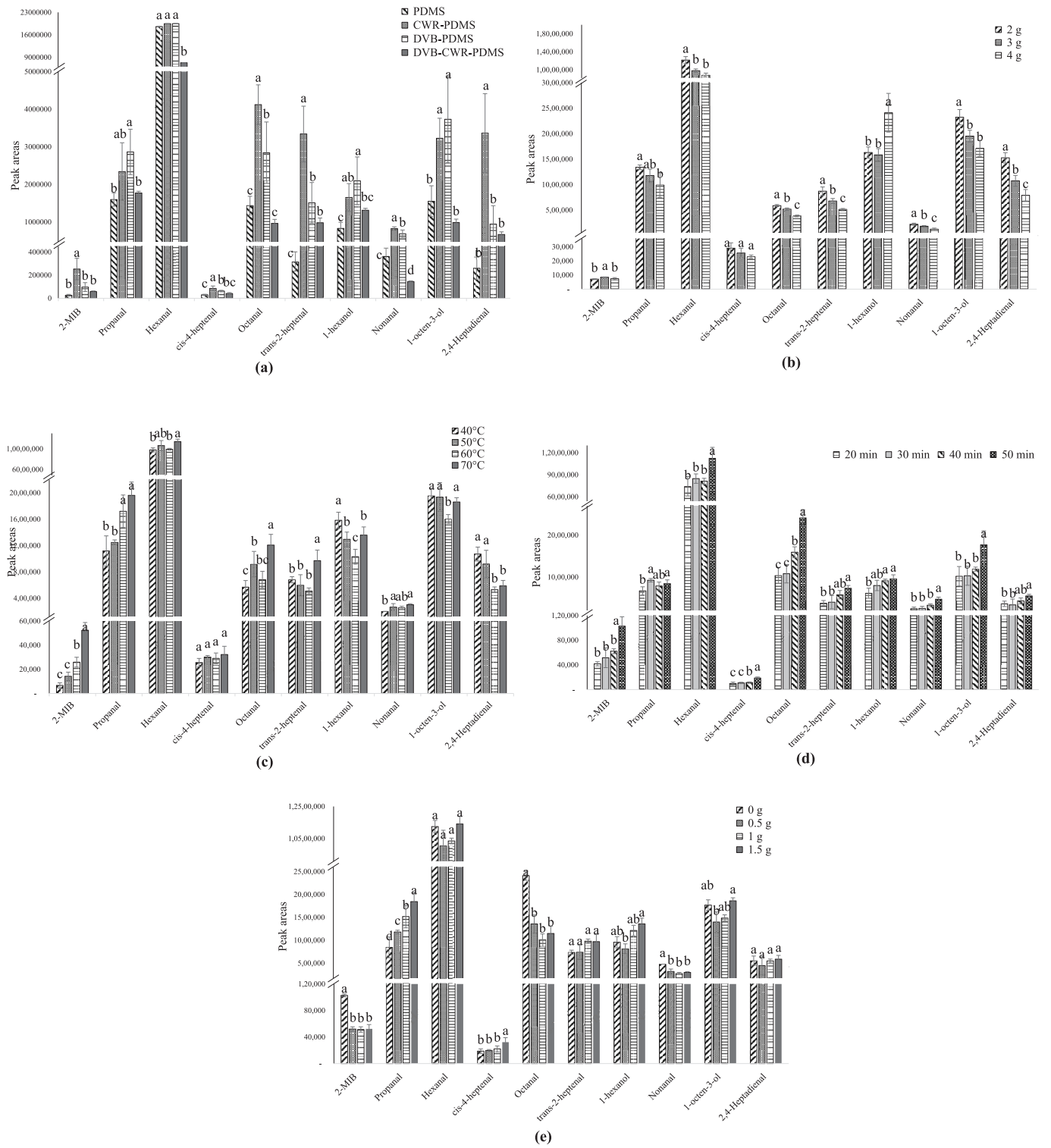

Fig. 1. Effects of (a) fiber, (b) fish weight, (c) extraction temperature, (d) extraction time, and (e) NaCl addition on SPME efficiency of volatile compounds in catfish mince. The standard deviation from triplicate determinations is represented by the bars. Different letters imply a statistically significant difference ($p < 0.05$).

might have occurred in our analyses when the sample solution became more concentrated as evidenced by the highest 4 g fish weight, which provided an unusually high RSD of 1-hexanol (16 %).

In contrast to the mentioned lipid oxidation-derived volatile compounds, 2-MIB gave the best extraction yield when using 3 g fish; at 2 g the low peak area of 2-MIB was a major concern. As a compromise, since the peak areas of the other volatile compounds was sufficient for reliable quantification also at 3 g fish, this sample weight was used to secure 2-MIB quantification as this is the most potent off-odor compound even at ultra-low levels (below ppm).

3.1.3. *Extraction temperatures*

The highest temperature used in this study provided the best extraction of 2-MIB, propanal, hexanal, octanal and *trans*-2-heptenal ($p < 0.05$) (Fig. 1c). This is most likely explained by the semi-volatile nature of 2-MIB. While, theoretically, higher temperatures contribute to

faster mass transport rates of semi- and low-volatile analytes into the headspace, volatile analytes can easily and naturally partition into the headspace already at room temperature [23]. According to this concept, as the temperature increases, the fluctuating and decreasing levels of the other assayed off-odor compounds could be due to their competition for fiber adsorption space with the large number of analytes that require high temperature to volatilize. To find a balance between volatilization and competition for fiber space, a maximum temperature of 70 °C was chosen as the optimal temperature to detect the semi-volatile 2-MIB, which provided the lowest peak areas in this study.

3.1.4. Extraction times

The effect of extraction time on the extraction of off-odor compounds from the catfish matrix is shown in Fig. 1d. The extraction of 2-MIB, hexanal, *cis*-4-heptenal, octanal, *trans*-2-heptenal, nonanal, 1-octen-3-ol, and 2,4-heptadienal, was clearly highest with the longest extraction time (50 min) ($p < 0.05$). This agrees with earlier findings from a spiked water solution, where the longer the extraction time, the higher the concentration of semi-volatiles in the gaseous phase [23,24]. According to our results, 50 min provided a satisfactory peak area response for most assayed off-odor compounds, why it was chosen as a suitable extraction time in this study.

3.1.5. Effect of salt additions

3.1.5.1. Salt amounts. Adding salt can release volatile molecules from the aqueous phase into the headspace via a salting out effect [25]. According to our results, NaCl addition aided extraction of four out of the ten assayed volatile compounds, i.e., propanal, *cis*-4-heptenal, 1-hexanol, and 1-octen-3-ol (Fig. 1e). Propanal extraction was most closely related to NaCl addition and was released proportionally to the tested NaCl concentration (Fig. 1e). This finding might be linked to the high polarity and thus low octanol partition coefficients (log $P = 0.60$) of propanal as salt addition particularly promotes extraction of polar compounds [23]. As the ionic salt molecules attract water molecules, their presence in the aqueous sample solution prevents water molecules from interacting with the analytes [26]. For the other volatile compounds which responded positively to salt, 1.5 g NaCl (30 % saturated NaCl) was required for maximum extraction (Fig. 1e). Similarly, Xue et al. [27] reported that the release of hexanal and 1-octen-3-ol from myofibrillar protein peaked at the highest tested ionic strength (1 M). The ionic strengths tested in this study were approximate 4.28, 8.56 and 12.83 M when using 0.5, 1, and 1.5 g NaCl/5 mL sample solution.

Our study further found that NaCl had no effect on the extraction of hexanal, *trans*-2-heptenal, and 2,4-heptadienal ($p < 0.05$). Unexpectedly, the extraction of 2-MIB, octanal, and nonanal was significantly limited by the presence of salt, with 2-MIB being 2 times lower. This phenomenon might be explained by the hydrophobicity of these analytes. Log P of 2-MIB, octanal, and nonanal was 3.31, 2.70, and 3.30, respectively, and these molecules were thus the most hydrophobic compared to other assayed compounds. The high ionic strengths evaluated in this study (4.28–12.83 M) could induce conformal changes of proteins, which in turn could release lipids from the catfish tissue. Thus, the retention of the more hydrophobic analytes (2-MIB, octanal, and nonanal) in the sample solution could be induced by lipid coalescence decreasing the lipid surface area (Fig. 1e). Further, Xue et al. [27] reported that high ionic strength (>1 M NaCl) could limit the interaction between flavor compounds and silver carp myofibrillar proteins due to their competition with charged amino acid side chain residues.

It was concluded from the performed tests with different amounts of salt that extracting 2-MIB without NaCl addition would be the best approach since it is present at ultra-low levels. However, it was hypothesized that a long holding time in the sample rack without NaCl-addition to maximize release of 2-MIB may result in underestimation and/or overestimation of lipid oxidation-derived aldehydes since they may bind to the sample matrix at the same time as new lipid oxidation may occur. To investigate this hypothesis, the salt-subjection time was investigated.

3.1.5.2. Time of salt subjection. To address the role of salt-subjecting time on oxidation-derived volatiles, different holding times in the sample rack (0, 4, 8, 12, 16, and 24 h), with and without NaCl, were investigated before the extraction step started (Fig. 2a-j).

As predicted, without NaCl, the response of all assayed volatile compounds, except, 1-hexanol, decreased with extended holding time. After 24 h holding, propanal, hexanal, octanal, and 2, 4-heptadienal had lost >70 % of their peak area response. Pignoli, Bou, Rodriguez-Estrada, and Decker [28] postulated that the lower extraction of hexanal and propanal from washed turkey muscle mince into the headspace was due to interaction with hydrophobic parts of the protein (hexanal) and entrapment into protein α-helixes and binding to free amino- and SH-groups (propanal). Additionally, Chaijan, Benjakul, Visessanguan, Lee and Faustman [29] reported that the binding of aldehydic lipid oxidation products (hexanal and hexenal) could alter the protein structure, leading to increased exposure of reactive groups. Thus, the more aldehyde-protein binding, the more protein unfolding would take place and thereby the exposure of hydrophobic surface would increase. This would then raise the aldehyde-protein binding over time and would restrict the role of the aldehyde as an active aroma provider.

As stated in the previous section, more stable quantification was found over time with NaCl added compared to without it. In line with this, after 24 h of holding in presence of NaCl, the highest loss of 2-MIB peak area was 42 %, which was a lower loss compared to without NaCl (68 % loss). Thus, both our results and those of other authors, e.g., Xue et al. [27], support that NaCl addition aids volatile compound analysis.

With more than 8 h holding time with NaCl, the sharp decrease in 2-MIB peak area and the drastically increased peak area of 1-hexanol indicated underestimation and overestimation of these volatiles respectively. Since the effect on other volatiles was minor, holding times of less than 8 h are suggested before the extraction step.

3.2. Validation parameters

Validation parameters were tested under the selected conditions as lined out above. In short, these were a CWR/PDMS fiber, 3 g fish mince diluted to 5 mL with DDW, 1.5 g added NaCl, volatile compound extraction at 70 °C/50 min and maximum 8 h holding of the sample rack before the extraction step. Table 2 lists all validation parameters for each addressed off-odor compound, including, linear range, R^2, LOD, LOQ, intraday variation (repeatability), interday variation (reproducibility), and recovery. All assayed analytes showed high linearity (0.9829–0.9963), except for the highly volatile propanal which only had a linearity of 0.9646. The LOD of 2-MIB (0.28 ng/g) yielded by our method was about 1.8 times lower compared to what was reported when the traditionally used HS-SPME-GC–MS method was applied to European whitefish *Coregonus lavaretus* (Table 1) (0.5 ng/g) [15]. Thus, the method presented here had higher sensitivity for the semi-volatile 2-MIB when analyzed in a fish matrix. Regarding the lipid oxidation-derived volatiles, the LOD was generally found to be lower than the actual levels found in the fish samples, especially the 0-day sample according to our previous study [6]. The repeatability and reproducibility ranked from 5 to 12 % RSD. The highest RSD for reproducibility, 12 %, was found for 2-MIB. When the recovery of each assayed volatile compound from catfish muscle matrix was tested at the lowest, middle, and highest concentration of the linear range, recoveries between 94 and 112 % were obtained.

3.3. Application of the method to iced stored fish muscle

The selected conditions were applied to analyze the targeted volatile

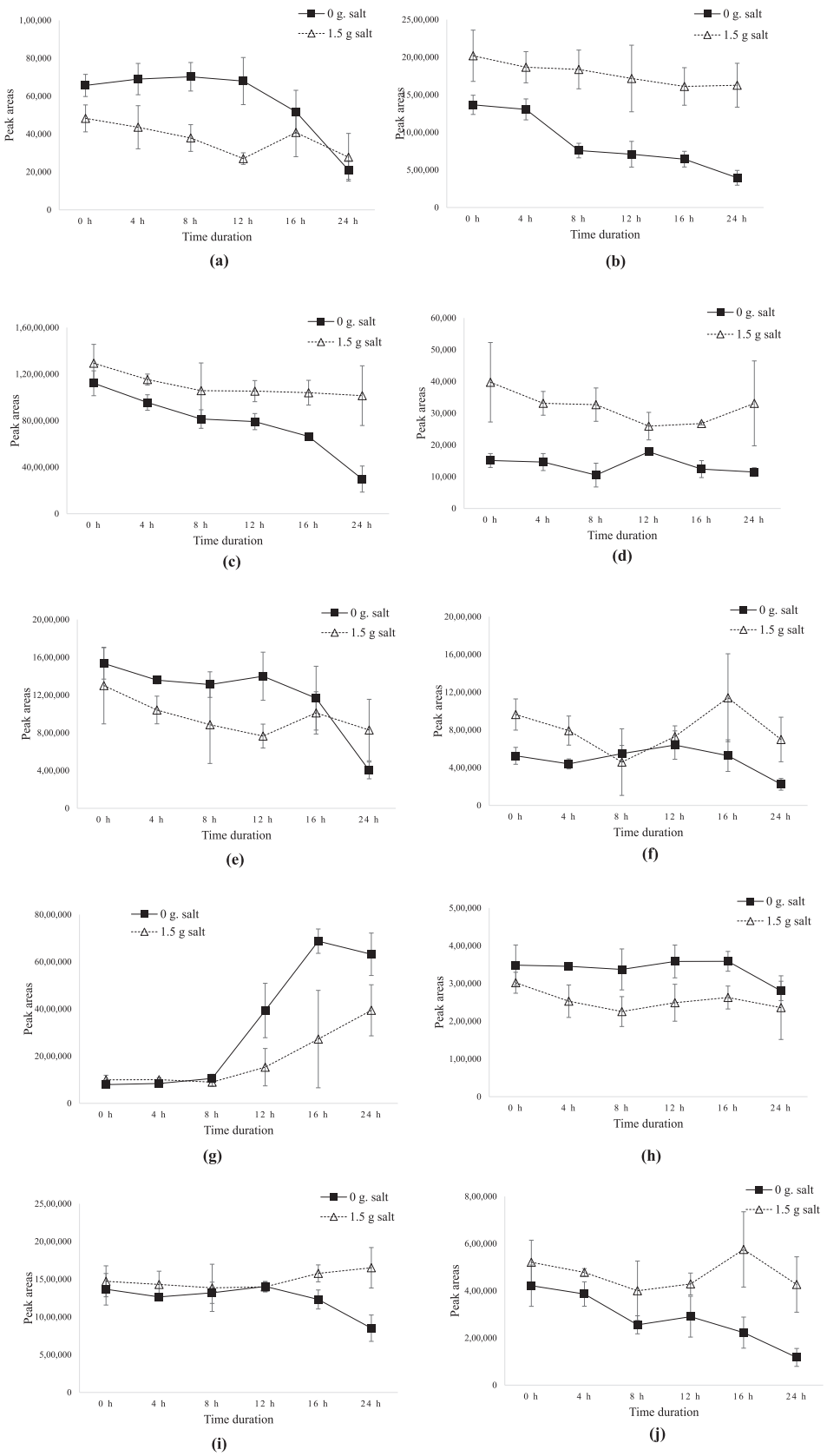

Fig. 2. The extraction efficiency of with and without salting-out effect during different time waiting (0, 4, 8, 12, 16, and 24 h) on total peak areas of (a) 2-MIB, (b) propanal, (c) hexanal, (d) *cis*-4-heptenal, (e) octanal, (f) *trans*-2-heptenal, (g) 1-hexanol, (h) nonanal, (i) 1-octen-3-ol and (j) 2,4-heptadienal.

compounds in dorsal, lateral line, and ventral muscle of catfish during 15 days of ice storage (Table 3). In parallel to these analyses, TBARS was also followed on the same samples since this method for measuring carbonyls, including MDA, is one of the most common strategies for investigating lipid oxidation products in muscle [30,31]. TBARS utilizes spectrophotometry to measure the pink adduct formed when TBA reacts with carbonyls. However, the TBARS method has been criticized for being non-specific and for being dependent e.g., on the amount of iron in the muscle samples [32,33], restricting comparisons between different types of tissues [30]. Despite the mentioned drawback, Pignoli et al. [28] found that TBARS was a more sensitive and accurate marker of lipid oxidation in washed turkey muscle compared to headspace techniques. We also earlier reported a strong correlation between TBARS and rancid odor development in Hb-fortified washed cod mince [34].

The outcome of the correlation analysis of all assayed volatile compounds with TBARS is shown in Table 3. 2,4-Heptadienal ($r = 0.774$) had the significantly highest positive correlation with TBARS, followed by hexanal ($r = 0.631$), *trans*-2-heptenal ($r = 0.627$), and propanal ($r = 0.512$). Thus, there was a moderate positive correlation between the two methods ($r = 0.51$ to $r = 0.80$) reflecting that they only partly capture the same lipid oxidation markers, but not completely. The latter aligns with the fact that TBARS do not specifically respond to volatile carbonyls, but to all carbonyls. Furthermore, the correlation analysis revealed that 2,4-heptadienal, hexanal, *trans*-2-heptenal, and propanal are specifically promising lipid oxidation markers when applying headspace analysis to catfish muscle. 2,4-Heptadienal has earlier been reported to be a good lipid oxidation marker in omega-3 rich fish oil [35] and ensilaged herring by-products [36]. The ice storage results from this study correlated well with our previous storage study carried out at 4 °C, where we found that propanal and hexanal were appropriate lipid oxidation markers for farmed hybrid catfish muscle [6]. Typically, hexanal and propanal are utilized as lipid oxidation indicators when applying headspace techniques, which is due to their stable structure explained by the lack of double bonds [37]. However, propanal is also important as oxidation marker since it can strongly influence the off-odor of fish due to its low odor-threshold (0.69 ppb).

4. Conclusion

For the first time, an SPME-Arrow technique was applied to enrich trace-levels of 2-MIB from fish and lipid oxidation products for specifically hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*), to facilitate their quantification. Fiber type, sample weight, NaCl addition, holding time before extraction, extraction time and extraction temperature were studied in a univariate approach. The selected conditions comprised a CWR-PDMS Arrow fiber, a small sample amount (3 g fish), 1.5 g added NaCl, a maximum holding time of 8 h in the sample rack before the extraction step to avoid under or over estimation of volatile lipid oxidation products followed by extraction at 70 °C/50 min. The LOD of 2-MIB (0.28 ng/g) in the presented method was lower compared to what has been reported from traditional HS-SPME-GC–MS methods. All volatile lipid oxidation products had good sensitivity; LOD was lower than what has earlier been observed in fresh fish samples. Moreover, when the method was applied to different types of catfish muscle stored for different amounts of time on ice, there was a relatively good correlation of the volatile aldehydes/alcohols, particularly 2,4-heptadienal, hexanal, *trans*-2-heptenal, and propanal with TBARS. Thus, altogether, this method showed strength to monitor precursors of both earthy/muddy and rancid odors in fish.

Ethical statement

This study did not include any human subjects and animal experiments.

Table 3
Correlation between assayed volatile compounds and TBARS in dorsal, lateral line and ventral muscle of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) during 15 days of iced storage.

Volatile compounds	TBARS
propanal	0.512*
hexanal	0.631**
cis-4-heptenal	−0.213
octanal	−0.216
trans-2-heptenal	0.627**
1-hexanol	0.155
nonanal	−0.002
1-octen-3-ol	0.440
2,4-heptadienal	0.774**

**Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

CRediT authorship contribution statement

Hatairad Phetsang: Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Data curation, Conceptualization. **Worawan Panpipat:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Manat Chaijan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Atikorn Panya:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Methodology, Investigation, Data curation. **Ingrid Undeland:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

Authors would like to declare that they do not have any conflict of interest.

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