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Analytical Methods

Rapid detection of oilfish and escolar in fish steaks: A tool to prevent keriorrhea episodes

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Abstract

The outbreak of keriorrhea caused by the wax ester-rich oilfish and escolar has become a frequent and worldwide concern. To help prevent such episodes, rapid detection of these fishes in the supply chain of the seafood industry and by food and health inspection agencies is essential. Through a combination of DNA, GC–MS and TLC analyses with reference to authentic samples, fish steaks of oilfish and escolar mislabeled as other species could be accurately identified. The TLC method developed is inexpensive and provides a reliable and importantly rapid identification within 30 min.

Keywords: Keriorrhea; Oilfish; Escolar; Diarrhea; Forensics; Wax ester

1. Introduction

Oilfish (*Ruvettus pretiosus*) and escolar (*Lepidocybium flavobrunneum*) belong to the Gempylidae (snake mackerel) family, and are the only species of their respective genera (Alexander et al., 2004). They have long been known to possess purgative effects because of their high oil content, accounting for approximately 20% of their wet weight (Cox & Reid, 1932; Mori, Saito, Nakanishi, Miyazawa, & Hashimoto, 1966; Ukishima et al., 1987). The major component contributing more than 90% of the total oil is indigestible wax esters, which cause diarrhea and other acute gastrointestinal symptoms, such as abdominal cramps, nausea, headache and vomiting (Alexander et al., 2004; Ruiz-Gutierrez, Perez-Zarza, Muriana, & Bravo, 1997; Yohannes, Dalton, Halliday, Unicomb, & Kirk, 2002). Although the aftermath is mild in most cases, consumption of oilfish or escolar by people with bowel sensitivity or by pregnant women could be risky (Shadbolt, Kirk, & Roche, 2002). Diarrhea associated with oilfish or escolar consumption is characterized by excretion of an orange to brown oil without causing loss of body fluid as in ordinary diarrhea; the term keriorrhea was coined for this type of diarrhea (Berman, Harley, & Spark, 1981). Long-term consumption of wax esters in mice could lead to seborrhea, in which wax esters were released through the sebaceous glands on the skin, blocking pores and potentially interfering with metabolism (Mori et al., 1966). Therefore, wax esters in these two fishes are regarded as a natural toxin called gempylotoxin (Food & Drug Administration, 2001a).

Outbreaks of keriorrhea associated with consumption of oilfish or escolar have been repeatedly reported in several continents (Berman et al., 1981; Shadbolt et al., 2002; Feldman et al., 2005; Gregory, 2002; Leask, Yankos, & Ferson, 2004; Givney, 2002). Many countries have introduced legislation or regulation on these two fishes. Italy and Japan

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banned their import and sale (Alexander et al., 2004). Australia, Canada, USA, and a majority of the member states of the European Union have issued special guidelines towards trading and consumption of these fishes.

An outbreak of over 600 cases of keriorrhea occurred in Hong Kong towards the end of 2006 (Chong, 2007; Chung, 2007a; Connolly, Wong, & Fong, 2007). Packages of oilfish steaks were mislabeled as codfish (Chong, 2007). Escolar, on the other hand, was found offered as sushi or sashimi under the name of snowfish or white tuna (Chung, 2007b; Mok, 2007). In February 2007, similar fish steaks were found in the Chinatown in Toronto, Canada, and resulted in an outbreak of keriorrhea. The oilfish outbreak awoke the Hong Kong public to food safety with strong demands for regulating and identifying these two fishes (Goh, 2007).

A global ban on oilfish and escolar trading is impossible as various countries have different policies; yet the two fishes are considered by many as undesirable for consumption. Where consumption occurs, suitable guidelines or advice to consumers are needed. Misidentification and mislabeling could happen throughout the entire supply chain, from fish importers, wholesalers, retailers to restaurants. The public and law-enforcement agencies require a convenient method for identifying the two fishes, together with a well-established labeling system. Various methods have been developed for seafood authentication over the years, including protein and DNA analyses (Civera, 2003). One study written in Japanese used SDS-polyacrylamide gel electrophoresis (SDS-PAGE) to differentiate oilfish and escolar from other commonly marketed fishes (Ochiai et al., 1984). However, if fishes are processed by cooking or sun-drying, the species-specific proteins could be denatured (Carrera et al., 1999). DNA-based techniques are less affected by food processing, even for canned tuna (Quinteiro et al., 1998). Thus, it would be preferable to utilize DNA analysis rather than protein analysis for identifying seafood (Bartlett & Davidson, 1992). DNA-based techniques, however, are expensive and time-consuming.

We combined DNA sequencing, GC–MS and TLC to identify oilfish and escolar and the wax esters extracted from them. A TLC method was developed for rapid differentiation of oilfish and escolar from other commonly marketed fishes, to provide food-control officers and members of the seafood industry a suitable protocol to rapidly detect the two fishes.

2. Materials and methods

2.1. Fish samples

Twenty-eight fish steaks or fillet samples were purchased from local markets in Hong Kong (Table 1). Voucher specimens of the 28 fish steaks were stored at −80 °C at the Chinese University of Hong Kong. Skin, including the outer lipid layer, was excluded and only meat was used. Six authentic reference samples were obtained from CSIRO Marine Laboratories, Australian National Fish Collection, including samples A1–A3 being *R. pretiosus* with voucher numbers H4215-02, Unregistered and H5147-01 as well as samples A4–A6 being *L. flavobrunneum* with voucher numbers H4782-01, H52332-01 and Unregistered, respectively. The six authentic samples were used to confirm the identities of marketed samples by DNA analyses. Fish sample R1 was cooked by steaming at 100 °C for 10 min or frying at 250 °C for 5 min; these samples were labeled as R1s (steamed) and R1f (fried), respectively.

2.2. DNA analysis

2.2.1. Extraction of DNA from meat

Four hundred microlitre of extraction buffer [200 mM Tris–HCl (pH 8.0), 200 mM NaCl, 25 mM EDTA, 0.5% SDS] and proteinase K(10 mg/ml) were added to 0.5 g of meat. The sample was cut into fine pieces. The solution was mixed and incubated at 37 °C for 1 h. 400 μl of 2X CTAB solution [2% CTAB (w/v), 100 mM Tris–HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 1% PVP (polyvinylpyrrolidone) Mr 40,000] was added and mixed gently. Proteins and lipids were extracted using chloroform: isoamyl alcohol (24:1) with 5% phenol (1:1, v:v). After centrifugation at 13,200*g* for 10 min, the aqueous phase was transferred to a microcentrifuge tube. The extraction process was repeated with an equal volume of chloroform:isoamyl alcohol (24:1). Two/third volume of isopropanol was added to the aqueous phase and the solution was mixed gently and incubated at room temperature for 10 min. The solution was spun at 13,200*g* for 10 min and the pellet washed with 70% ethanol. The supernatant was discarded and the pellet dried at 60 °C for 10 min. DNA was resuspended in 30 μl double distilled water and stored at −20 °C.

2.2.2. Polymerase chain reaction and DNA sequencing

Polymerase chain reaction of the mitochondrial 12s rRNA gene, 16s rRNA gene, cytochrome *b* gene, and cytochrome oxidase subunit I (cox1) gene was performed in a 25 μl reaction containing 1 ng DNA, 1x *Taq* buffer [75 mM Tris–HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$ 0.01% Tween 20], 0.1 mM dNTPs, 1 unit of *Taq* polymerase and 1 μM of primers. The sequences of primers used for amplification were 12L1 (5′AAA AAG CTT CAA ACT GGG ATT AGA TAC CCC 3′) and 12H1 (5′TGA CTG CAG CAG AGG GTG ACG GGC GGT GTG T 3′) for 12s rRNA fragment, 16L1 (5′CTG ACC GTG CAA AGG TAG CGT AAT CAC T 3′) and 16H1 (5′CTC CGG TCT GAA CTC AGA TCA CGT AGG 3′) for 16s rRNA fragment (Hedges, 1994), L14841 (5′ ATC CAA CAT CTC AGC ATG ATG AAA 3′) and H15149b (5′ CCC CTC AGA ATG ATA TTT GTC TCA 3′) for cytochrome *b* fragment (Kocher et al., 1989), and FishF1 (5′ TCA ACC AAC ACA AAG ACA TTG GCA C 3′) and FishR1 (5′ TAG ACT TCT GGG TGG CCA AAG AAT CA 3′) for cox1 gene (Ward, Zemlak, Innes, Last,

Table 1
Identities of 28 fish steaks and 2 cooked samples based on BLAST search results from GenBank and BOLD

No.	Voucher codes	Commodity names*	Identities (common names)**	12s/16s/cyt *b* (GenBank sequence matched %)/cox1 (BOLD species similarity %)**	Correct label? (Y/N)
1	R1	Cod fish	*Ruvettus pretiosus* (oilfish)	16s (99%); cyt *b* (99%); cox1 (99.83%)	N
2	R2	Cod fish		16s (99%); cyt *b* (99%); cox1 (99.83%)	N
3	C3	Canada cod fish		16s (100%); cyt *b* (100%); cox1 (100%)	N
4	C4	Canada silver cod		16s (100%); cyt *b* (99%); cox1 (100%)	N
5	E1	Cod fish (oilfish, *Ruvettus pretiosus*)		16s (100%); cyt *b* (100%); cox1 (99.83%)	N
6	E2	White cod fish		16s (99%); cyt *b* (99%); cox1 (100%)	N
7	E3	Yellow cod fish		16s (99%); cyt *b* (100%); cox1 (100%)	N
8	E5	Snowfish	*Lepidocybium flavobrunneum* (escolar)	cyt *b* (97%); cox1 (99.66%)	N
9	E6	Snowfish		cyt *b* (97%); cox1 (99.83%)	N
10	E7	Snowfish		cyt *b* (97%); cox1 (99.66%)	N
11	R1f	=Fried R1	*Ruvettus pretiosus* (oilfish)	16s (99%); cyt *b* (99%); cox1 (99.83%)	N
12	C1	Canada black cod	*Anoplopoma fimbria* (black cod)	16s (99%); cox1 (100%)	Y
13	C2	Canada black cod	*Anoplopoma fimbria* (black cod)	16s (100%); cox1 (100%)	Y
14	B1	South Africa sea bass	*Dissostichus mawsoni* (sea bass)	12s (100%); 16s (99%); cox1 (100%)	Y
15	E4	Silver cod fish	*Reinhardtius hippoglossoides* (halibut)	12s (100%); cox1 (99.49%)	N
16	C5	Canada black cod	*Anoplopoma fimbria* (black cod)	16s (100%); cox1 (100%)	Y
17	T1	Russia salmon	*Oncorhynchus mykiss* (rainbow trout)	cox1 (100%)	Y
18	T2	Salmon	*Oncorhynchus keta* (salmon)	12s (100%)	Y
19	G2	New Zealand ling	*Genypterus blacodes* (ling)	cox1 (99.32%)	Y
20	S2	Vietnam catfish	*Pangasianodon hypophthalmus* (catfish)	12s (99%); 16s (98%); cyt *b* (99%); cox1 (100%)	Y
21	M1	Monkfish	*Kathetostoma giganteum* (monkfish)	cyt *b* (100%); cox1 (100%)	Y
22	W1	Philippines swordfish	*Xiphias gladius* (swordfish)	cyt *b* (100%); cox1 (100%)	Y
23	G3	Mackerel fish	*Eleutheronema tetradactylum*	16s (98%); cox1 (96.36%)	Y
24	G1	Silver cod fish	*Gadus morhua* (Atlantic cod)	16s (99%); cox1 (97.12%)	Y
25	B2	Chilean sea bass	*Dissostichus eleginoides* (Chilean sea bass)	12s (99%); 16s (100%); cox1 (100%)	Y
26	H2	Norwegian halibut	*Reinhardtius hippoglossoides* (halibut)	12s (100%); cox1 (99.66%)	Y
27	H1	Greenland halibut	*Reinhardtius hippoglossoides* (halibut)	12s (100%); cox1 (100%)	Y
28	J1	Leather jacket	*Aluterus monoceros* (leather jacket)	cox1 (100%)	Y
29	S1	Sole fillet (Vietnam)	*Pangasianodon hypophthalmus* (catfish)	12s (99%); 16s (99%); cyt *b* (99%); cox1 (100%)	Y
30	R1s	=Steamed R1	*Ruvettus pretiosus* (oilfish)	16s (99%); cyt *b* (99%); cox1 (99.83%)	N

* The labels shown on the fish steak packages.
** Results based on BLAST search for 12s rRNA, 16s rRNA and cyt *b* genes in GenBank and for cox1 gene in BOLD. Common names based on FishBase.

& Hebert, 2005). The primers flanked sites 1087–1478 for 12L1/12H1, sites 2607–3055 for 16L1/16H1, and sites 14841–15149 with reference to human mitochondrial genome (Hedges, 1994; Kocher et al., 1989). Initial template denaturation temperature was 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min.

The PCR products were purified from TAE (Tris-acetate-EDTA) agarose gel using VIOGENE Gel-M™ Gel Extraction System (Cat. No.: EG1002) following the manufacturer's directions. The purified DNA was sequenced by Sanger's Dideoxy method using ABI3100 DNA sequencer following the manufacturer's directions.

2.2.3. Sequence analysis

Alignments of the mitochondrial 12s rRNA gene, 16s rRNA gene, cytochrome *b* gene and cytochrome oxidase subunit I (cox1) gene were accomplished with the computer program BioEdit version 7.0.5.3. Dendrograms were constructed using MEGA version 3.1 (Kumar, Tamura, &

Nei, 2004) with neighbor-joining method and a Kimura-two parameter (K2P) distance model. Confidence in dendrograms was assessed by the bootstrap method, with 1000 replications. BLAST searches revealed the identities of samples, with GenBank used for the mitochondrial 12s rRNA gene, 16s rRNA gene and cytochrome *b* gene; and barcode of life database (BOLD, www.barcodeoflife.org) for cox1 gene.

2.3. Gas chromatography–Mass spectrometry (GC–MS) analysis

2.3.1. Sample preparation and lipid extraction

Fish samples (4 g in wet weight) were cut into fine pieces. Lipid was extracted with 6 ml hexane using an ultrasonic vibrator (Branson 3200, USA) with gentle stirring and squeezing for 5 min. After centrifugation at 13,200*g* for 2 min, the supernatant was transferred to new tube.

2.3.2. GC–MS analysis

GC–MS analysis of the supernatant was performed on a J&W fused silica capillary column (30 m, 0.25 mm ID, 0.25 μm film thickness) in a Shimadzu GCMS-QP2010 GC–MS System with oven temperature programming, 100 °C, 1 min; 100 °C to 300 °C, rate 3 °C/min; 300 °C, 40 min. Injector temperature was 280 °C and transfer line temperature was 280 °C with ion source temperature 200 °C. Helium (purity = 99.999%) was the carrier gas, linear velocity on column 36.7 cm/s. MS analysis was performed in a quadrupole mass spectrometer, using electron impact ionization mode. Electron energy was −70 eV, ion polarity was positive, mass range was 50–1000 a.m.u., scan time was 0.5 s and the electron multiplier voltage was 1000 V.

2.4. Thin-layer chromatography (TLC) analysis

2.4.1. Chromatogram development and wax ester detection

Supernatant was applied to silica gel plates (Silica gel 60F254, Merck) using disposable micropipettes. Plates were developed in a glass tank lined with filter paper and saturated with xylene, which was the mobile phase for resolving non-polar lipids. After development, the plate was oven-dried, sprayed with 40% sulfuric acid in ethanol:anisaldehyde (9:0:1), and heated at 100 °C for 2 min or until color was observed. Another TLC plate was developed as above, with no spray used. Translucent spots on the plates were extracted with 3 ml acetone for GC–MS analysis.

3. Results

3.1. Identification by DNA analysis

PCR amplifications of the four mitochondrial gene regions were successful for all 34 samples, and for the two cooked oilfish R1s and R1f. The read lengths for mitochondrial 12s rRNA gene, 16s rRNA gene, cytochrome *b* gene and cox1 gene were about 400 bp, 500 bp, 300 bp and 600 bp long respectively. Twenty-four DNA sequences of the four genes in the six authentic reference samples (A1–A6) were deposited in GenBank with accession numbers ranging from EU003538 to EU003561. Sequences of the four mitochondrial gene regions were used to construct four neighbor-joining (NJ) trees (Fig. 1, only 12s rRNA gene sequence tree is shown). All NJ trees clearly revealed that some samples (C3, C4, E1, E2, E3, E5, E6, E7, R1 and R2) retailed as "cod fish" or "snowfish" were actually oilfish or escolar and their sequences clustered with those of authentic samples.

Oilfish and escolar are closely related species belonging to the same family. This relationship was shown in the NJ trees where both species clustered in a well defined group and separated from other commonly marketed fishes (Fig. 1). Clustering of oilfish and escolar were supported by high bootstrap values and thus, DNA analysis can be used to differentiate oilfish and escolar from other fishes.

DNA sequences of the four mitochondrial gene regions were blast-searched in GenBank and BOLD to confirm the identities of the 28 marketed fish samples (Table 1). It was found that seven fish samples (C3, C4, E1, E2, E3, R1 and R2), which were labeled as codfish, silver cod, yellow cod or white cod, matched with oilfish. Amongst these seven samples, only E1 bore "oilfish" as supplementary information on the package. Another three, E5, E6 and E7, labeled as snowfish, were escolar. Sample E4, which was sold as silver cod, was identified as halibut, as it clustered to both halibut samples H1 and H2 (Fig. 1). Only one sample, G1, was genuine cod fish, which belongs to the genus *Gadus*.

Cooked oilfish R1s and R1f had the exact sequences to the untreated sample, R1, for all four mitochondrial gene regions; yet moderate DNA fragmentation was observed in the DNA extraction gel (data not shown). Cooking processes, such as steaming and frying, did not completely degrade the DNA required for proper authentication in this case.

3.2. GC–MS and TLC analyses

Lipid extracts of all samples were analyzed by GC–MS. Characteristic peaks showing the existence of wax esters were found only in samples identified by DNA sequences as oilfish and escolar (Fig. 2A). Utilization of the computerized NIST Library Search program compared the sample mass spectra to the database of standard mass spectra; compounds exhibiting the most prominent peaks (Peaks 1–4 in Fig. 2A) were closely matched with unsaturated wax esters containing 32–36 carbons. Cooked oilfish samples (Rls and R1f) showed identical patterns to untreated oilfish samples (Fig. 2B). All other fishes did not display wax esters and showed similar GC–MS profiles (Fig. 2C).

Lipid extracts from the fish samples also were used for TLC analysis. A characteristic spot at Rf = 0.6 was found only in oilfish and escolar (Fig. 3). The two cooked oilfish samples (R1s and R1f) showed identical TLC band patterns to the untreated oilfish and escolar. In contrast, other

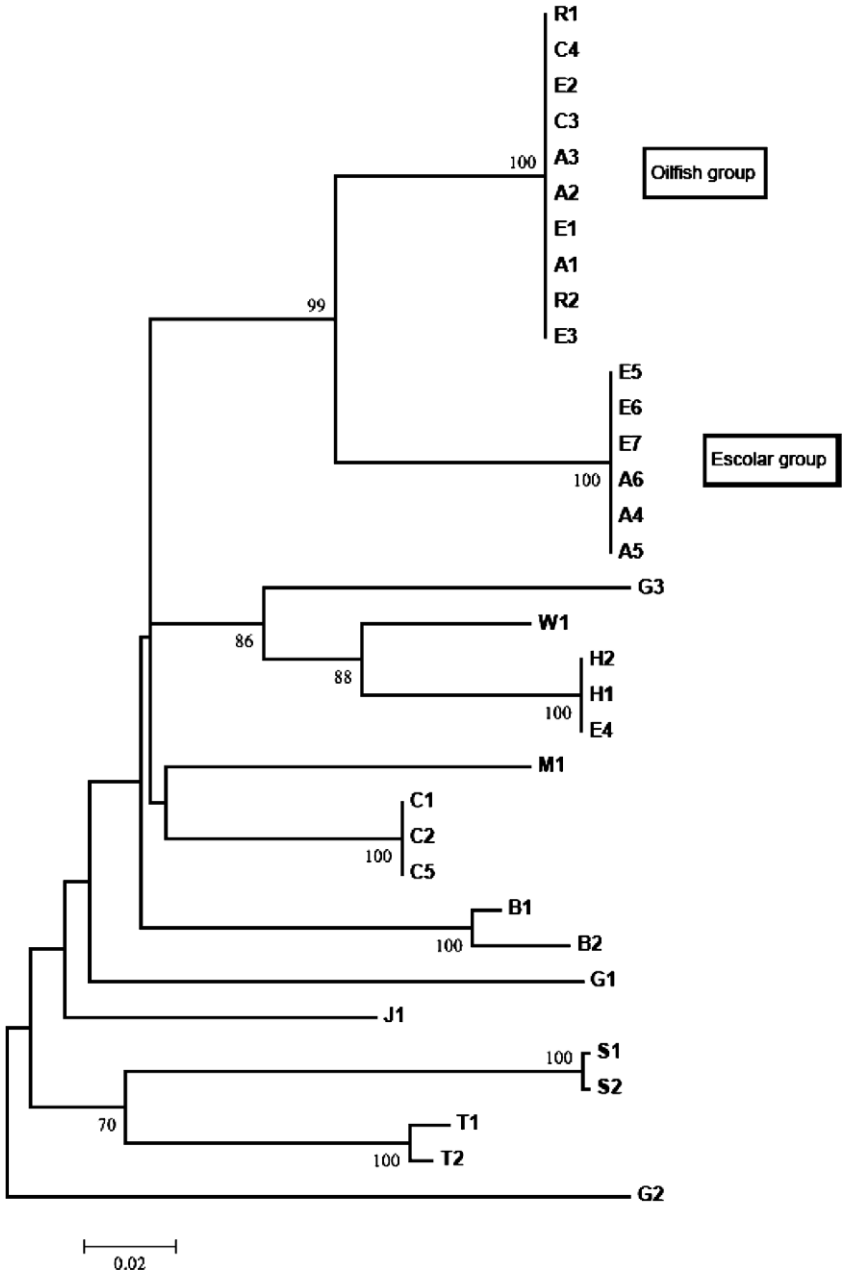

Fig. 1. K2P distance neighbor-joining tree of 12s rRNA gene sequences from 34 fish samples (codes refer to Table 1). Samples that were identified as oilfish and escolar clustered into their own groups; with bootstrap values indicated at branches (bootstrap values less than 50 are not shown).

commonly marketed fishes displayed no spot at $Rf = 0.6$, meaning that these heat stable and low polarity lipids exist only in oilfish and escolar, and not in other tested fishes. GC–MS analysis of the acetone extract of the spot at $Rf = 0.6$ confirmed that it consisted of a mixture of wax esters (data not shown).

4. Discussion

4.1. Confusing labeling system in the markets

Fishery products are generally processed with the external features eliminated. In our study, the fish steaks or fillets were cut in a way that only a small portion of the skin remained attached to the flesh, while in samples E4, E5 and E6, they were sold as sashimi without skin. Our results showed that DNA authentication enabled species identification of these fish products, even for the cooked samples R1f and R1s.

DNA analysis revealed substantial confusion in fish labeling. Mislabeling and substitution are worldwide concerns, and can be intentional or accidental. In this study, the name "cod" was found to be used widely and intentionally to label fish species of very different families. True cod belongs to the genus *Gadus* with only three species. Two of

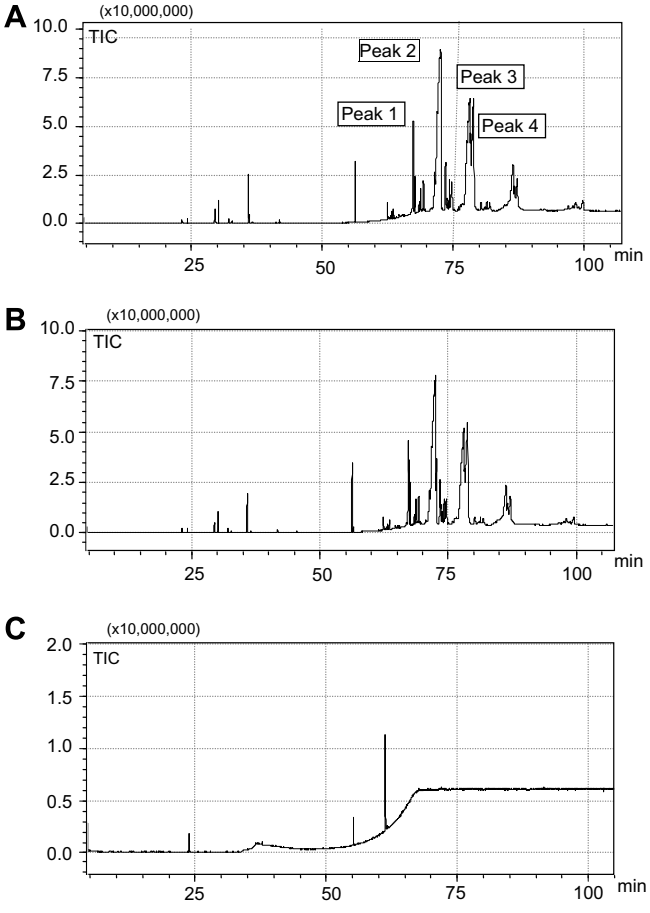

Fig. 2. GC–MS total ion chromatograms for (A) oilfish sample R1, (B) cooked oilfish sample R1s and (C) black cod sample C2. Peak identification by NIST Mass Spectral Library Search. Peak 1, r.t.: 67.4 min, 9-octadecenoic acid (*Z*)-, tetradecyl ester, CAS no.: 22393-85-7, MW: 478, similarity index: 93. Peak 2, r.t.: 72.7 min, 9-octadecenoic acid (*Z*)-, hexadecyl ester, CAS no.: 22393-86-8, MW: 506, similarity index: 93. Peak 3, r.t.: 78.3 min, 9-octadecenoic acid (*Z*)-, 9-octadecenyl ester, (*Z*)-, CAS no.: 3687-45-4, MW: 532, similarity index: 92. Peak 4, r.t.: 78.9 min, 9-octadecenoic acid (*Z*)-, octadecyl ester, CAS no.: 17673-49-3, MW: 534, similarity index: 93.

the three species, *Gadus morhua* (Atlantic cod) and *G. macrocephalus* (Pacific cod), are commercially important but overfishing has caused dramatic declines in stocks over the years, such that *G. morhua* is now considered a vulnerable species (IUCN Red List of Threatened Species, 2006). The limited supply of cod has driven the substitution of expensive cod by halibut, sea bass or oilfish as in the keriorrhea outbreak in Hong Kong. Accidental mislabeling also contributed to several outbreaks of keriorrhea associated with oilfish or escolar consumption in Australia and the US (Shadbolt et al., 2002; Feldman et al., 2005; Gregory, 2002; Leask et al., 2004; Givney, 2002). In those cases, oilfish or escolar were mislabeled as rudderfish, butterfish, ruddercod, mackerel, walu or gemfish. A consistent and legally-enforceable labeling system is required to ensure safety of fish consumption and DNA analysis should be part of such a system.

Although DNA analysis is accurate to the species level and can be used for cooked samples, it is time-consuming and expensive. In addition, DNA fragmentation during cooking could prevent successful amplification of a large size gene region. For differentiating oilfish and escolar from other common fishes, TLC analysis is faster and simpler and may be preferred.

4.2. TLC – a convenient way to detect oilfish and escolar

Wax esters, consisting of a fatty acid esterified to a fatty alcohol, provide buoyancy in oilfish and escolar. Unlike many heat-labile proteins, wax esters are heat stable and indigestible by humans, and pass through the digestive tract. It is this stability characteristic that makes the use of wax esters for identification purposes reliable, even on cooked and digested fish samples. Wax esters are very low polarity lipids and can be easily distinguished by TLC from other lipids, such as triglycerides. Wax esters are more stable than most proteins and also vary less among different tissues. A previous study used soluble muscle proteins from oilfish and escolar for identification (Ochiai et al., 1984) and was useful only for muscle. However, wax esters are present in different muscle parts, such as dorsal, ventral, subcutan and periosteum; and various internal organs, including liver, gall bladder and testes, of oilfish (Ruiz-Gutierrez et al., 1997). Therefore, the detection of wax esters for identification has advantages over protein profiling in terms of stability and consistency across tissues.

In this study, TLC succeeded in rapidly differentiating oilfish and escolar from other fishes. The whole process, from extraction to staining, took less than 30 min while the equipment involved is readily available in most laboratories. Each TLC plate can accommodate more than 20 samples, and multiple plates can be run simultaneously. It is a space-saving and suitable technique for screening large sample numbers.

4.3. Wax esters and deep-water fishes

Major lipid components of most fish species, including commercially important fishes, are triglycerides and phospholipids. Wax esters, in contrast, are less common lipids and, where they occur in deep-water fishes, provide a way to enhance buoyancy (Bone, 1972). In deep-water fishes, such as oilfish, escolar, orange roughy (*Hoplostethus atlanticus*), species of oreos (*Allocyttus* sp., *Pseudocyttus* sp., *Neocyttus* sp. and *Oreosome* sp.) and myctophid fishes (*Lampanyctus ritteri*, *Stenobrachius leucopsarus* and *Tryphoturus mexicanus*), wax esters predominate in the lipid (Bakes, Elliott, Green, & Nichols, 1995; Nevenzel, Rodegker, Robinson, & Kayama, 1969; Phleger, Nelson, Mooney, & Nichols, 1999; Rehbein et al., 1995). Although not all wax ester-rich fishes were investigated here, with the exception of orange roughy (*H. atlanticus*), most of these deep-water species are not widely distributed and seldom

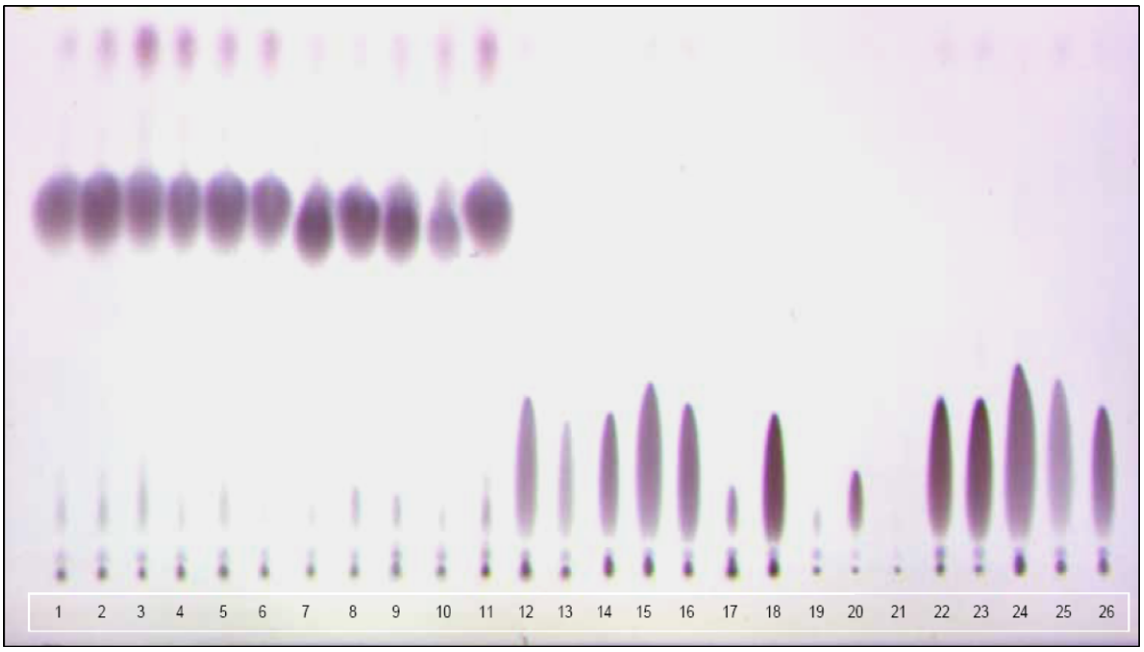

Fig. 3. Thin-layer chromatogram of 26 samples observed under visible light (lane sequence and sample codes follow those in Table 1). Only oilfish and escolar samples (lanes 1–11) showed a characteristic spot at Rf = 0.6.

marketed in quantity. On the other hand, oilfish and escolar are widely distributed in tropical and temperate seas, comparatively abundant and marketed as a result of their by-catch with tuna and swordfish (Tserpes, Tatamanidis, & Peristeraki, 2006). Up to 400 tonnes of escolar are caught annually in Australia (Shadbolt et al., 2002). In addition, those mentioned deep-water fishes, except oilfish and escolar, are small in size according to FishBase records (www.fishbase.org), thus reducing the likelihood that these fishes are substituted as cod steaks, which are usually larger. Oilfish and escolar are of comparable size to cod and sea bass, making them an ideal substitution. For deep-water species that have wax esters as their main lipid, the composition of wax esters are not identical in terms of carbon chain length and double bond number (Bakes et al., 1995; Mori et al., 1966; Nevenzel et al., 1969; Nichols, Mooney, & Elliott, 2001; Phleger et al., 1999; Reinhardt & Van-Vleet, 1986), thus, allowing differentiation of oilfish and escolar from other wax ester-rich fishes by GC–MS.

4.4. Recommendations

A complete ban on import and selling of oilfish and escolar would ensure no mis-consumption of these species. A proper authentication method is definitely needed with this measure to check for substitution, misidentification and mislabeling. In countries where trading of oilfish and escolar is legal, labels should be accompanied by a warning sign indicating the potential adverse effects and providing guidelines for handling.

Although not all wax ester-rich fish species were tested in this study, further purification of the wax esters (gempylotoxin) can be used as a standard marker for authentication of wax ester containing species. By running the samples to be tested together with the standard marker on the same TLC plate, the existence of undesirable wax esters can be detected and DNA analysis or GC–MS analysis can be performed later to confirm identity. The problem for regulating oilfish and escolar is that not all people who consume these fishes develop the symptoms. Individual differences in body sensitivities, health conditions, intake amounts and other factors may contribute to variations in susceptibility. From case reports, it is not possible to establish an intake level which is considered safe from adverse effect (Alexander et al., 2004). The best way is to detect for wax ester, as the presence of this lipid class may be a sign for problems, and it is not recommended to serve any flesh that contains high levels of wax esters before a thorough inspection is done. TLC together with the standard marker presents a rapid way for routine inspection to safeguard the public.

Besides wax ester poisoning, oilfish and escolar are common candidates for histamine poisoning, which has a greater affect than keriorrhea and can be life-threatening. Oilfish and escolar contain high levels of histidine in muscle (Feldman et al., 2005). If these fishes are inadequately refrigerated, bacteria can multiple and convert histidine into histamine, also termed scombrotoxin (Food & Drug Administration, 2001b). This conversion often happens when large numbers of unsold fish steaks were stocked over time to avoid food inspection in case of a related keriorrhea outbreak. The fish may re-appear later in markets, but at that time, the steaks may be contaminated and not suitable

for consumption. Scombrotoxin, like wax esters, is heat stable and is not destroyed by cooking; it can lead to cardiovascular, gastrointestinal and neurological symptoms (FDA, 2001b; Feldman et al., 2005; Leask et al., 2004). Therefore, detection of wax esters could help prevent more severe food poisoning from happening once any keriorrhea outbreak is reported.

5. Conclusions

Intentional or unintentional supply and offer of oilfish and escolar very often leads to episodes of unpleasant keriorrhea. Time is the essence for rapid detection of these fishes rich in wax ester for food and health inspection agencies. Equally important for the seafood industry supply chain is a convenient measure to check for the two fishes. In routine food inspection of large numbers of fish samples, cost, speed and accuracy are important for species authentication. The TLC method described is inexpensive and provides a reliable result within 30 min. This method can be performed routinely to check for substitution of fish products with either oilfish or escolar. In the case of keriorrhea resulting from consumption of mislabeled oilfish or escolar, the TLC method can be applied even on the residues of cooked samples as evidence for species identification.

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