

Multiplex PCR detection of mackerel-based food adulteration with pleco and chicken in selected areas around Ciliwung River, Indonesia

RINI WIDAYANTI¹, HERJUNO ARI NUGROHO², DOROTHEA VERA MEGARANI¹,
DYAH AYU WIDIASIH¹, SUHENDRA PAKPAHAN^{2,✉}

¹Faculty of Veterinary Medicine, Universitas Gadjah Mada. Jl. Fauna No. 2, Sleman 55281, Yogyakarta, Indonesia

²Research Center for Applied Zoology, National Research and Innovation Agency. Jl. Raya Jakarta-Bogor Km 46, Cibinong, Bogor 16911, West Java, Indonesia. ✉email: suhendra.pakpahan@brin.go.id

Manuscript received: 29 November 2022. Revision accepted: 8 March 2023.

Abstract. Widiyanti R, Nugroho HA, Megarani DV, Widiasih DA, Pakpahan S. 2023. Multiplex PCR detection of mackerel-based food adulteration with pleco and chicken in selected areas around Ciliwung River, Indonesia. *Biodiversitas* 24: 1538-1543. Detecting fish product adulteration is crucial to ensure food safety since pleco meat was already reported to carry several heavy metals that might harm human health. Pleco is invasive species in the Ciliwung River and is commonly used as adulteration material for fish-based products. Adulteration in mackerel-based food products may alter the nutritional value and carry heavy metal contamination from the bottom-feeder fish's meat (pleco). Therefore, using the DNA barcoding technique, a molecular approach has been used to authenticate mackerel fish products (including dumplings and *otak-otak*). This study aimed to develop a specific multiplex PCR method for simultaneously detecting processed products from mackerel and pleco. The sample consists of 21 processed food items initially made from mackerel. The samples were taken in the selected area around the Ciliwung River. All the samples can be amplified successfully, and amplification lengths were 108, 171, and 300 bp, respectively. Analysis from various claimed mackerel products showed that five samples were positive for pleco adulteration, and 11 products contained chicken meat addition. The phylogenetic tree was constructed from selected sequences from our samples and showed that the amplicons were clustered in three clades, mackerel (*Scomberomorus*), pleco (*Pterygoplichthys*), and chicken (*Gallus gallus* Linnaeus, 1758). The findings of this study revealed that 23.80% (5/21) products contained pleco, and 52.38% (11/21) contained chicken meat addition. The addition of an unusual component to food composition may alter nutritional value as well as may affect food hygiene and safety.

Keywords: Ciliwung River, food adulteration, mackerel, multiplex PCR, pleco

INTRODUCTION

As a maritime country, Indonesia has an enormous fishery production potential. The potential wealth of genetic resources in land and sea areas is significant, as Indonesia's marine area covers 70% of the total area. This vast area holds great potential, including the supply of food sources, such as mackerel (*Scomberomorus* sp.). Fishery products can be an alternative source of protein apart from the livestock sector. One of the potential fishery products from Indonesia is mackerel, a species of marine fish with a particular flavor that is popular in Indonesian society. This pelagic fish is found in almost all marine areas of Indonesia (Widayanti et al. 2022). Mackerel in Indonesia has been processed into many local foods such as dumplings, *otak-otak*, and *empek-empek*. This food is widely consumed, especially in educational environments such as schools and colleges, because it is relatively cheap.

Food made from mackerel has grown in popularity in Indonesian society. Unfortunately, the counterfeiting of raw materials threatens this relatively affordable food. One of the raw materials used to adulterate mackerel fish is pleco (*Hypostomus plecostomus* Linnaeus, 1758) and *Pterygoplichthys pardalis* Castelnau, 1855 (Nurjanah et al. 2015). Therefore, to gain economic benefits, the most common methods of adulteration in Brazil were deliberate

dilution and replacement (Tibola et al. 2018). Identifying the provenance of animal species in processed meat products is critical for economic, religious, and hygienic reasons. Meat products frequently suffer from species substitutions and adulterations due to their high market value (Cawthorn et al. 2013). Concerns about food safety and regulation have prompted the development of several approaches for detecting food adulteration. Those include physical, biochemical/immunological, and molecular techniques. Molecular approaches are preferred for detecting biological adulterants in food (Bansal et al. 2017).

Pleco was originally imported to Indonesia as an ornamental fish. This fish is imported from South America and breeds rapidly in the Indonesian river environment (Armbruster and Page 2006; Munandar 2016). This fish is currently considered an invasive species in several rivers across the globe, including several rivers in Jakarta's area, like the Ciliwung River (Elfidasari et al. 2018). Two species of plecos were reported from the Ciliwung River, including *P. pardalis* and *Pterygoplichthys disjunctivus* Weber, 1991 (Elfidasari et al. 2016). The abundance of this fish as an invasive species is one reason to use this fish to adulterate processed mackerel fish (Elfidasari et al. 2019). Pleco fish was reportedly harvested for meat in Jakarta (Ismi et al. 2019). Pleco's meat contains 45-50% protein, 1.11-1.12% fats, 0.2-1.15% ash, and 47-52% carbohydrates

(Elfidasari et al. 2019). However, the fishbone from pleco's meat processing is unsuitable for bone flour since it does not meet the minimum nutritional value required by the Indonesian National Standard (SNI) (Putri et al. 2020).

Pleco has a high tolerance for living in a polluted environment. This fish's bottom-feeding behavior can potentially accumulate heavy metal contamination in its meat. Heavy metal pollutants such as arsenic (As), Cadmium (Cd), Mercury (Hg), and Lead (Pb) can accumulate in the bodies of fish that inhabit polluted areas. Heavy metal contamination in pleco has been reported from various regions. Such as the pleco from the Bengawan Solo River, which Cu, Cd, and Pb polluted (Setyarini et al. 2005), contamination by Pb and Cd from Fish from Bedadung River, Jember (Munandar 2016), and fish from the Ciliwung River, Jabodetabek, Indonesia which Cd, Hg polluted, and Pb (Elfidasari et al. 2018). Therefore, pleco's meat is not feasible for consumption because of the high concentration of Pb, Hg, and Cd that exceeds the maximum value of the SNI threshold (Ismi et al. 2019). Heavy metal contamination in food products in the long and short term can cause various diseases, such as neurological diseases to congenital disabilities in infants. The existing research on food adulteration reveals fraudulent replacement and mislabeling of meat and other food products (Quinto et al. 2016).

Molecular techniques are used to study the diversity of mackerel species in Indonesia and to authenticate mackerel fish products (*bakso*, *empek-empek*, and crackers) using a DNA barcoding technique that targets the Cytochrome b gene (Maulid and Nurilmala 2015). In addition, several methods to detect meat product adulteration have been developed during the last two decades (Ali et al. 2014; Sentandreu and Sentandreu 2014). Recently, several studies have focused on species-specific PCR that targets the amplification of mitochondrial genes (Alikord et al. 2016; Al-taghlubee et al. 2019; Qin et al. 2019; Dwiyoitno et al. 2022). This study aims to develop a specific multiplex PCR method for simultaneously detecting processed products from mackerel and pleco. This study also examines the adulteration of processed mackerel-based food products with other meats, especially pleco, in Jakarta, Depok, and Bogor, areas where the Ciliwung River passes. We assume that adulteration occurs in areas that the river passes through.

MATERIALS AND METHODS

Sample collection

Sample collection, a total of 21 commercial food products made from mackerel were collected from several locations in Jakarta, Depok, and Bogor, Indonesia (Table 1). Purposive sampling was used as the selected areas passed by the Ciliwung River. All processed mackerel food samples were preserved using RNAlater and stored in the laboratory.

Fresh samples were promptly extracted for DNA. Before extraction, RNAlater®-preserved samples were frozen and centrifuged at 5000 G for 10 minutes, with the

supernatant discarded. Next, to eliminate excess salt from the RNAlater® solution, samples were washed three times with a sterile phosphate buffer solution. Finally, each sample was homogenized independently for 2 minutes before being cut into 100 mg for the next DNA extraction, using a tissue homogenizer.

Molecular techniques

Total DNA was extracted from the homogenized food product samples. Isolation and purification of DNA using DNA Isolation Kit (innuPREP) following the manufacturer's procedures. The isolated DNA was stored at -20°C before being used for the next step. Following the manufacturer's procedures, total DNA was extracted from the homogenized food product samples using a DNA isolation kit (innuPREP). The isolated DNA was then stored at -20°C before being used for the next step. The primers were designed using the 3output primer program (http://www.genome.wi.mit.edu/cgi-bin/primer3.cgi/results_from-primer3). DNA reference sequences for mackerel, pleco, and chicken were obtained from the NCBI database. The genes that are the target of amplification are the mitochondrial Cyt B gene and 12 rRNA (Table 2). The primers were designed using the Primer3 program (http://www.genome.wi.mit.edu/cgi-bin/primer3.cgi/results_from-primer3). DNA reference sequences for mackerel, pleco, and chicken were obtained from the NCBI database. The genes targeted for amplification were the mitochondrial Cyt B gene and 12S rRNA.

Table 1. Commercial food products from mackerel and sampling locations

Code	Processed food from mackerel	Location
JKU1	Dumpling	Tamansari, Jakarta
JKU2	Dumpling	Tamansari, Jakarta
JKU3	Dumpling	Tamansari, Jakarta
JKU4	Dumpling	Tamansari, Jakarta
D1	<i>Otak-otak</i>	Beji, Depok
D2	Dumpling	Beji, Depok
D3	Dumpling	Beji, Depok
BO1	Dumpling	Pasar Anyar, Cibogor, Bogor
BO2	Dumpling	Pasar Anyar, Cibogor, Bogor
BO3	Dumpling	Pasar Anyar, Cibogor, Bogor
BO4	<i>Batagor</i>	Pasar Anyar, Cibogor, Bogor
PR	Dumpling	Jl. Pandu Raya, Tegal Gundil, Bogor Utara, Bogor
BS	Dumpling	Jl. Raya Bogor-Sukabumi, Baranangsiang, Bogor
ES	<i>Empek-empek</i>	Suryakencana, Bogor
OS	<i>Otak-otak</i>	Suryakencana, Bogor
RD	Dumpling	Radio Dalam, Gandaria Utara, Cilindak, Jakarta
CR	Dumpling	Jl. Ciputat Raya, Pondok Pinang, Kebayoran Lama, Jakarta
GI	Dumpling	Grand Indonesia, Menteng, Jakarta
CBN	Dumpling	Cibinong, Bogor
JKM1	Dumpling	Manggarai, Jakarta
JKM2	Dumpling	Manggarai, Jakarta

Table 2. Primary design that will be used to distinguish the processed products of mackerel and pleco

Target gene	Nucleotide sequence	Amplicon
Cyt B	F:5'TATAGTAGTCCCATTCCTTCAC 3'	108 bp
<i>Scomberomorus</i> sp.	R:5'CAGGTTAGAATTGCTACGTCT 3'	
12SrRNA pleco	F: 5' TTACCAAATATGCCCGACT 3'	171 bp
<i>Gallus gallus</i>	R: 5' GCTCCTCTAGGTGGGTCT 3'	300 bp

The total volume of the PCR reagents was 50 µL, consisting of 25 µL of master mix, 2 µL of DNA template, 1 µL (10 pmol) of each primer, and 21 µL of ddH₂O. Mitochondrial DNA taken from four different types of food was combined and used as templates in the same proportion (10 ng each). Two control positives were made from pure meat of mackerel and pleco, respectively. The following amplifications were performed under optimal PCR conditions: Predenaturation: at 94°C 5 minutes, denaturation at 94°C 30 seconds, annealing at 53°C 30 seconds, elongation at 72°C 30 seconds, and final elongation at 72°C 5 minutes and 40 cycles. The PCR amplification results were electrophoresed using 2.5% agarose gel. Genotyping analysis was carried out based on the length of the bp bands on the agarose gel, mackerel 108 bp; pleco 171 bp, and chicken 300 bp. Sequencing was performed on partial positive findings, and the results were consistent with the multiplex PCR results.

RESULTS AND DISCUSSION

Results

All samples were successfully amplified, and the electrophoresis results indicated amplification lengths of 108, 171, and 300 bp, respectively (Figure 1). The study samples were identified and compared with the positive (mackerel) and the negative samples. The samples were divided into three groups based on the target amplification of the primers used: mackerel only, mackerel combined with pleco and chicken, and mackerel combined with chicken (Table 3). Analyzing all varieties of processed mackerel food revealed that 23.80% (5/21) of products contained pleco, and 52.38% (11/21) contained chicken meat. Pleco-containing products originated from many sources that provided a variety of foods from different locations, such as dumplings, *otak-otak*, and *batagor*. The processed dumplings types with mackerel ingredients without mixing are only 29%, with additional pleco ingredients at 6%, additional chicken ingredients at 47%, and a mixture of pleco and chicken at 12% (Figure 2). The phylogenetic tree was constructed from selected sequences from our samples and showed that the amplicons were clustered into three clades with bootstrap values of 92-100 (Figure 3). Several representative samples were chosen to continue to the sequencing step to validate the genotyping results based on the genotyping result. These clades correspond to three amplicons produced in PCR using our designed primer set. The mackerel amplicon was 108 bp, pleco 171 bp, and chicken 300 bp, respectively. The *Scomberomorus commerson* Lacepède, 1800 (mackerel)

clade is represented by samples BO2, D1, and D3; *P. pardalis* (pleco) is represented by samples D1, JKU4, and BO2; and *Gallus gallus* Linnaeus, 1758 (chicken) is represented by sample PR.

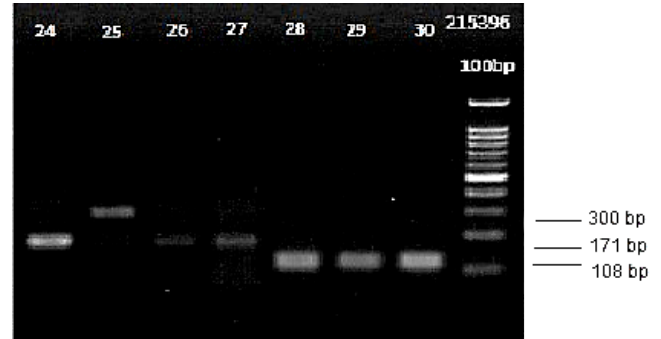


Figure 1. Internal reference primers amplified the bands. M: marker 100 bp; lanes 24 to 30 represent PCR products: mackerel 108 bp; pleco 171 bp; and chicken 300 bp, respectively

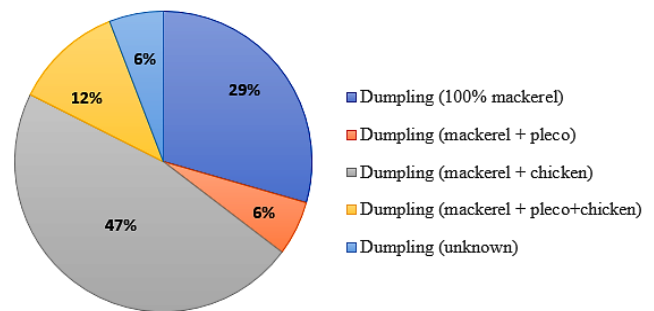


Figure 2. Dumpling ingredients (mackerel, pleco, and chicken)

Table 3. Identify the components of the samples (mackerel, pleco, and chicken)

Sample code	Products of mackerel	Mackerel	Pleco	Chicken
T (positive control)	Dumpling	+	-	-
S (positive control)	Dumpling	-	+	-
JKU1	Dumpling	+	-	+
JKU2	Dumpling	+	-	+
JKU3	Dumpling	+	-	-
JKU4	Dumpling	+	+	+
D1	<i>Otak-otak</i>	+	+	-
D2	Dumpling	+	-	+
D3	Dumpling	+	-	-
BO1	Dumpling	+	-	-
BO2	Dumpling	+	+	+
BO3	Dumpling	+	-	+
BO4	<i>Batagor</i>	+	+	+
PR	Dumpling	+	-	+
BS	Dumpling	+	-	+
ES	<i>Empek-empek</i>	+	-	-
OS	<i>Otak-otak</i>	+	-	-
RD	Dumpling	+	-	+
CR	Dumpling	+	-	+
GI	Dumpling	+	-	-
CBN	Dumpling	+	-	-
JKM1	Dumpling	+	+	-
JKM2	Dumpling	-	-	-



Figure 3. The phylogenetic tree was constructed from selected sequences from our samples. It showed that the amplicons were clustered in three clades: mackerel (*S. commerson*), pleco (*P. pardalis*), and chicken (*G. gallus*)

Discussion

Based on our samples, 4 of 21 were adulterated with a pleco. The adulterated products were randomly found in some sampling sites in Jakarta (1/9), Depok (1/3), and Bogor (2/9). Unexpectedly, chicken meat addition was also found in several mackerel-based products in Jakarta (5/7), Depok (1/3), and Bogor (5/9). We assume this addition enhances palatability or increases protein content since mackerel stock may be scarce in the market. However, pleco adulteration needs more concern since it may harbor heavy metal accumulation. We also found that 1 sample did not contain mackerel, pleco, or chicken addition. Moreover, for economic reasons, plecostomus from the Ciliwung River is utilized as the main ingredient for culinary items such as *dim sum*, *otak-otak*, *empek-empek*, and fish chips (Nitibaskara and Madiah 2005). One of the invasive fish in Indonesian waterways is the *H. plecostomus*, often known locally as *sapu-sapu* fish. This fish's presence has a variety of effects on the river environment. The Ciliwung River is now highly polluted in every area, from the upstream (Bogor District) to the downstream (Jakarta City), making it unsafe to use for recreational purposes (Elfidasari et al. 2018). *H. plecostomus* can survive in extreme conditions of heavy metal contamination (Cano-Rocabayera et al. 2022), making it the dominant species in the Ciliwung River. The heavy metals Pb, Hg, and Cd were detected in *H. plecostomus* (Loricariidae) from the Ciliwung River using the XRF technique. Since heavy metal concentrations exceed the SNI standard's maximum value, plecostomus meat from the Ciliwung River in Jakarta is unsafe for human consumption (Ismi et al. 2019).

Adulteration is the addition of unusual components to food that can alter the nutritional value and affect food hygiene and safety (Kowalska et al. 2019). Adulteration is a common problem in Indonesia, as evidenced by

examining commercial food products made from mackerel in the marketplace. These findings demonstrate the actual occurrence of adulteration in commercial food products made from mackerel in the marketplace. The added other components in food are usually considered unacceptable by society. Therefore, multiplex PCR has become increasingly popular because of its ease of use, sensitivity, accuracy, cost-effectiveness, and potential for high-throughput benefits. This approach will be highly beneficial if it can detect the types of commercial food ingredients that are often altered. Liu et al. (2019) developed and optimized nine distinct multiplex PCR primers based on the sequence variance of specific mitochondrial genes. Four different meat products were rapidly detected by electrophoresis using an optimized multiplex PCR apparatus based on the molecular weight variations of the amplified products. The detection sensitivity could reach 0.05 ng/ μ L. Adulteration of fox, raccoon, or mink in retail, commercial beef, and mutton meat has become a severe concern. Li et al. (2019) created a multiplex PCR approach to detect fox, mink, or raccoon components in beef and mutton meat at 1% (w/w).

The selection of target genes was critical to creating the multiple PCR system due to the significant similarity among diverse commercial mackerel food sources. The fish mtDNA has a wide range of intraspecific and interspecific polymorphisms, which allows for identifying closely related species. Because animal species' mitochondrial DNA (mtDNA) sequences, such as the 12S rRNA and Cyt B, are so highly conserved, they are usually applied as templates in the design of PCR identification techniques (Rojas et al. 2011). The length and high mutation rate of the mtDNA 12S rRNA gene made it a suitable molecular marker for distinguishing meat species, resulting in considerable genetic interspecies variability and making it simpler to distinguish between closely related species (Rojas et al. 2008; Galal-Khallaf 2021). Mitochondrial DNA provides certain benefits over nuclear DNA for processed meat identification, including the following: (i) stable; (ii) it contains many copies within the cell; and (iii) it has substantial inter-specific variability, allowing for accurate identification of all species under study (Rashid et al. 2015).

Various ways have been established to identify adulteration of commercial food components up to this point, including physical, chemical, biochemical, and molecular methods. The Loop-mediated isothermal amplification (LAMP) technique targeting species-specific mitochondrial DNA could identify and distinguish eight meat species: cow, pig, horse, goat, sheep, chicken, duck, and turkey. This assay was completed in 30 minutes and showed higher sensitivity than the PCR assays (Cho et al. 2014). The Hyperspectral Imaging (HSI) technique is an efficient, environmentally friendly, and non-destructive alternative for identifying and quantifying the fats of leaf lard adulteration in pork without prior physical or chemical analytical information (Jiang et al. 2020). Fourier transforms infrared (FTIR) spectroscopy using an attenuated total reflectance (ATR) approach can be used to evaluate the adulteration of beef meatballs with pork. The partial least squares method may be used successfully to

measure the degree of adulterant in pork in a specific fingerprint region (1200-1000 cm⁻¹) (Rohman et al. 2011). The enzyme immunoassay (ELISA, immune sensor) was successfully used for species screening tests and efficiently detected pork IgG in processed food. It can also identify pork in boiling beef meatballs in the examined range of 1 to 100% (Mandli et al. 2018).

Compared to immunological, chromatographic, and DNA hybridization methods for detecting meat adulteration, this PCR method was preferable, especially for highly processed meat products. It requires species-specific gene sequences, complex primer designs for multiple species, and stringent reaction optimization (Ali et al. 2014). In Indonesia's fresh waters, pleco is very abundant and not consumed. These less expensive foods are thus readily accessible in Indonesian marketplaces and frequently mixed into other food products to increase earnings. According to the study, the PCR technique is reliable for detecting adulteration-suspected DNA from mackerel, suckermouth, and chicken. Our findings suggest that pleco adulteration still occurred in several places in Jakarta, Depok, and Bogor areas. Our designed primer set could amplify pleco's genetic material contaminated with the mackerel-based product. We assumed that this addition increased the protein content in the products since mackerel may be scarce in the market, or it was cheaper for production cost by adding pleco's meat. Previous studies on pleco's uses in the Jakarta area showed that this bottom-feeder fish has lower nutritional value and may accumulate heavy metals from polluted habitats (Elfidasari et al. 2018). Pleco's meat addition to fish-based processed food might also risk human health. Future studies in a wider area, especially from the surrounding areas of the Ciliwung River route, must confirm our findings on a larger scale.

As an important environmental component, rivers, including economic growth, play a crucial role in human existence. The Ciliwung River in Indonesia flows through Bogor, Depok, Jakarta, and Bogor District, with a major flow length of more than 120 km. However, the water quality of the Ciliwung River and its surroundings has been steadily deteriorating due to increased population. The river serves numerous important purposes, including providing raw water for drinking, fishing, cattle and agriculture, and urban enterprises. In addition, the Ciliwung River is home to at least 20 freshwater fish species, with plecostomus being the dominant species (Hadiaty 2011). The Ciliwung River, Jakarta's largest city-bound river, was assessed using a mass flux technique. The simulated water quality indicated that the Ciliwung River basin was moderate to heavily contaminated. From the base year of 2000, the average degradation rates due to population expansion for NO₃, BOD, and COD are 53.03%, 14.10%, and 28.15%, respectively (Kumar et al. 2017). The first assessment found that the Ciliwung River transfers about 5-17 tons of defined pollutants into the Java Sea yearly (Dsikowitzky et al. 2018). The high pollution in the downstream area is caused by waste disposal from densely inhabited areas and diversified downstream activities. Among the pollutants, fecal coliform bacteria and total coliform cause the most pollution in the Ciliwung River (Ratnaningsih et al. 2019).

Heavy metals are one of the most dangerous components of river pollution for humans. Plecostomus from the Ciliwung River is frequently used as a major ingredient in culinary items. However, according to the National Standard of Indonesia (SNI), the Pb, Hg, and Cd concentrations in plecostomus meat are beyond the permissible range for consumption. Therefore, plecostomus from the Ciliwung River is unsuitable for human consumption (Elfidasari et al. 2018).

In Indonesia, there is no formal definition of food fraud or "economically motivated adulteration" (EMA) of foods. The Indonesian Food and Drug Authority (BPOM) still monitors food in the community. However, those processed mackerel products are typically prepared at home and sold directly to consumers; BPOM seldom identifies them. According to the results of the dumpling processed foods identification, only 29% use pure mackerel ingredient without combined with other ingredients such as pleco and chicken. Unsafe food will hamper the promotion of public health. Through an assessment of Indonesia's food safety system and the efficacy of law enforcement against hazardous food producers, an analysis of the effectiveness of law enforcement against hazardous food producers needed toward Healthy Indonesia 2025 is presented (Hung 2020). Most events remain unnoticed because they do not pose a food safety concern, and customers frequently do not notice a quality issue. Risk assessments for food safety, food defense, and food fraud analyze many criteria to establish the degree of situational risk for each category and the steps that must be taken to minimize that risk. More research is needed to help the government (food safety agency), which helps decrease overall risk even though the possible risks are unclear.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia) for financial support under grant No. 018/E5/PG.02.00/2022018, No. 018/E5/PG.02.00.PT/2022, and No. 1704/UN1/DITLIT/Dit-Lit/PT.01.03/2022. The authors also thank the Biochemistry and Molecular Biology Laboratory, Universitas Gadjah Mada, Indonesia assistants for their support.

REFERENCES

- Ali ME, Razzak MA, Hamid SBA. 2014. Multiplex PCR in species authentication: Probability and prospects-A review. *Food Anal Methods* 7: 1933-1949. DOI: 10.1007/s12161-014-9844-4.
- Alikord M, Keramat J, Kadivar M, Momtaz H, Eshtiaghi MN, Homayouni-Rad A. 2016. Multiplex-PCR as a rapid and sensitive method for identification of meat species in halal-meat products. *Recent Pat Food Nutr Agric* 8 (3): 175-182. DOI: 10.2174/2212798409666170113151213.
- Al-Taghlube D, Misaghi A, Shayan P, Akhondzadeh BA, Gandomi H, Shayan D. 2019. Comparison of two multiplex PCR systems for meat species authentication. *J Food Qual Hazards Control* 6 (1): 8-15. DOI: 10.18502/jfqc.6.1.453.
- Armbruster JW, Page LM. 2006. Redescription of *Pterygoplichthys punctatus* and description of a new species of *Pterygoplichthys*

- (Siluriformes: Loricariidae). *Neotrop Ichthyol* 4 (4): 401-409. DOI: 10.1590/S1679-62252006000400003.
- Bansal S, Singh A, Mangal M, Mangal AK, Kumar S. 2017. Food adulteration: Sources, health risks, and detection methods. *Crit Rev Food Sci Nutr* 57 (6): 1174-1189. DOI: 10.1080/10408398.2014.967834.
- Cano-Rocabayera O, Monroy M, Moncaleano-Niño ÁM, Gómez-Cubillos MC, Ahrens MJ. 2022. An integrated biomarker approach: Non-monotonic responses to cadmium exposure in the sucker-mouth catfish *Hypostomus plecostomus*. *Aquat Toxicol* 248: 106193. DOI: 10.1016/j.aquatox.2022.106193.
- Cawthorn DM, Steinman HA, Hoffman LC. 2013. A high incidence of species substitution and mislabelling detected in meat products sold in South Africa. *Food Control* 32 (2): 440-449. DOI: 10.1016/j.foodcont.2013.01.008.
- Cho AR, Dong HJ, Cho S. 2014. Meat species identification using loop-mediated isothermal amplification assay targeting species-specific mitochondrial DNA. *Korean J Food Sci Anim Resour* 34 (6): 799. DOI: 10.5851/kosfa.2014.34.6.799.
- Dsikowitzky L, Van der Wulp SA, Ariyani F, Hesse KJ, Damar A, Schwarzbauer J. 2018. Transport of pollution from the megacity Jakarta into the ocean: Insights from organic pollutant mass fluxes along the Ciliwung River. *Estuar Coast Shelf Sci* 215: 219-228. DOI: 10.1016/j.ecss.2018.10.017.
- Dwiyitno D, Hoffman S, Parmentier K, Keer CV. 2022. Universal primer design for crustacean and bivalve-mollusc authenticity based on cytochrome-b gene: Universal primer for crustacean and bivalve-mollusc. *Biodiversitas* 23 (1): 17-24. DOI: 10.13057/biodiv/d230103.
- Elfidasari D, Qoyyimah FD, Fahmi MR. 2016. Morphometric and meristic of common pleco (Loricariidae) on Ciliwung Riverwatershed South Jakarta Region. *Intl J Adv Res* 4 (11): 57-62. DOI: 10.21474/IJAR01/2363.
- Elfidasari D, Ismi LN, Shabira AP, Sugoro I. 2018. The correlation between heavy metal and nutrient content in *Pterygoplichthys pardalis* from Ciliwung River in Jakarta. *Biosaintifika* 10 (3): 597-604. DOI: 10.15294/biosaintifika.v10i3.16248.
- Elfidasari D, Shabira AP, Sugoro I, Ismi LN. 2019. The nutrient content of *Pterygoplichthys pardalis* flesh from Ciliwung River Jakarta, Indonesia. *Nusantara Biosci* 11 (1): 30-34. DOI: 10.13057/nusbiosci/n110106.
- Galal-Khallaif A. 2021. Multiplex PCR and 12S rRNA gene sequencing for detection of meat adulteration: A case study in the Egyptian markets. *Gene* 764: 145062. DOI: 10.1016/j.gene.2020.145062.
- Hadiaty RK. 2011. Diversitas dan hilangnya jenis-jenis ikan di Sungai Ciliwung dan Sungai Cisadane. *Berita Biologi* 10 (4): 491-504. DOI: 10.14203/beritabiologi.v10i4.767. [Indonesian]
- Hung MS. 2020. Efektivitas penegakan hukum terhadap produsen makanan berbahaya menuju Indonesia sehat 2025. *Jurnal Gagasan Hukum* 2 (2): 182-193. DOI: 10.31849/jgh.v2i02.8558. [Indonesian]
- Ismi LN, Elfidasari D, Puspitasari RL, Sugoro I, Sabira AP. 2019. The contents of heavy metals in *Plecostomus* (Loricariidae) from the Ciliwung river Jakarta, Indonesia. *Proceeding of International Biodiversity and Ecology Sciences Symposium*. Istanbul, Turkey, 26-28 September 2019.
- Jiang H, Jiang X, Ru Y, Wang J, Xu L, Zhou H. 2020. Application of hyperspectral imaging for detecting and visualizing leaf lard adulteration in minced pork. *Infrared Phys Technol* 110: 103467. DOI: 10.1016/j.infrared.2020.103467.
- Kowalska EH, Grela M, Gryzińska M, Listos P. 2019. Molecular techniques for detecting food adulteration. *Med Weter* 75 (7): 404-409. DOI: 10.21521/mw.6261.
- Kumar P, Masago Y, Mishra BK, Jalilov S, Rafiei EA, Kefi M, Fukushi K. 2017. Current assessment and future outlook for water resources considering climate change and a population burst: A case study of Ciliwung River, Jakarta City, Indonesia. *Water* 9 (6): 410-425. DOI: 10.3390/w9060410.
- Li J, Li J, Xu S, Xiong S, Yang J, Chen X, Wang S, Qiao X, Zhou T. 2019. A rapid and reliable multiplex PCR assay for simultaneous detection of fourteen animal species in two tubes. *Food Chem* 295: 395-402. DOI: 10.1016/j.foodchem.2019.05.112.
- Liu W, Wang X, Tao J, Xi B, Xue M, Sun W. 2019. A Multiplex PCR assay mediated by universal primers for the detection of adulterated meat in mutton. *J Food Prot* 82 (2): 325-330. DOI: 10.4315/0362-028X.JFP-18-302.
- Mandli J, Fatimi IE, Seddaoui N, Amine A. 2018. Enzyme immunoassay (ELISA/immunosensor) for a sensitive detection of pork adulteration in meat. *Food Chem* 255: 380-389. DOI: 10.1016/j.foodchem.2018.01.184.
- Maulid DY, Nurilmala M. 2015. DNA barcoding untuk autentifikasi produk ikan tenggiri (*Scomberomorus* sp.). *Jurnal Akuatika* (2): 154-160. [Indonesian]
- Munandar K. 2016. Kandungan logam berat Pb dan Cd pada ikan sapu-sapu yang tertangkap di Sungai Bedadung Kabupaten Jember. *Proceeding of National Seminary II Prodi Pendidikan Biologi FKIP dan Pusat Studi Lingkungan dan Kependudukan Universitas Muhammadiyah Malang*, 26 Maret 2016. [Indonesian]
- Nitibaskara, Madiyah. 2005. Pengaruh penambahan bahan pengikat terhadap karakteristik fisik otak-otak ikan sapu-sapu. *Jurnal Pengolahan Hasil Perikanan Indonesia* 8 (1): 1-11. DOI: 10.17844/jphpi.v8i1.1021. [Indonesian]
- Nurjanah, Suseno SH, Hidayat T, Ekawati Y, Paramudhita P, Arifianto. 2015. Change composition chemical of skipjack tuna due to frying process. *Intl Food Res J* 2 (5): 2093-2102.
- Putri HD, Elfidasari D, Haninah H, Sugoro I. 2020. Nutritional content of bone flour made from plecos fish *Pterygoplichthys pardalis* from the Ciliwung River, Indonesia. *Biosaintifika* 12 (3) (2020): 329-334.
- Qin P, Qu W, Xu J, Qiao D, Yao L, Xue F, Chen W. 2019. A sensitive multiplex PCR protocol for simultaneous detection of chicken, duck, and pork in beef samples. *J Food Sci Technol* 56 (3): 1266-1274. DOI: 10.1007/s13197-019-03591-2.
- Quinto CA, Tinoco R, Hellberg RS. 2016. DNA barcoding reveals mislabeling of game meat species on the US commercial market. *Food Control* 59: 386-392. DOI: 10.1016/j.foodcont.2015.05.043.
- Rashid NRA, Ali ME, Hamid SBA, Rahman MM, Razzak MA, Asing, Amin MA. 2015. A suitable method for the detection of a potential fraud of bringing macaque monkey meat into the food chain. *Food Addit Contam* 32 (7): 1013-1022. DOI: 10.1080/19440049.2015.1039073.
- Ratnaningsih D, Nasution EL, Wardhani NT, Pitalokasari OD, Fauzi R. 2019. Water pollution trends in Ciliwung River based on water quality parameters. *IOP Conf Ser Earth Environ Sci* 407: 012006. DOI: 10.1088/1755-1315/407/1/012006.
- Rohman A, Erwanto Y, Man YBC. 2011. Analysis of pork adulteration in beef meatball using Fourier transform infrared (FTIR) spectroscopy. *Meat Sci* 88 (1): 91-95. DOI: 10.1016/j.meatsci.2010.12.007.
- Rojas M, González I, Fajardo V, Martín I, Hernandez PE, García T, Martín R. 2008. Polymerase chain reaction–restriction fragment length polymorphism authentication of raw meats from game birds. *J AOAC Intl* 91 (6): 1416-1422. DOI: 10.1093/jaoac/91.6.1416.
- Rojas M, González I, Pavón MA, Pegels N, Hernández PE, García T, Martín R. 2011. Mitochondrial and nuclear markers for the authentication of partridge meat and the specific identification of red-legged partridge meat products by polymerase chain reaction. *Poultry Sci* 90 (1): 211-222. DOI: 10.3382/ps.2010-00895.
- Sentandreu MÁ, Sentandreu E. 2014. Authenticity of meat products: Tools against fraud. *Food Res Intl* 60: 19-29. DOI: 10.1016/j.foodres.2014.03.030.
- Setyarini RE, Astuti D, Ambarwati. 2005. Studi kandungan logam berat (Cu, Cd, Pb) pada ikan sapu-sapu (*Hypostomus plecostomus*) di Sungai Bengawan Solo. *Forum Geografi* 19 (2): 103-114. DOI: 10.23917/forgeo.v19i2.4840. [Indonesian]
- Tibola CS, da Silva SA, Dossa AA, Patrício DI. 2018. Economically motivated food fraud and adulteration in Brazil: Incidents and alternatives to minimize occurrence. *J Food Sci* 83 (8): 2028-2038. DOI: 10.1111/1750-3841.14279.
- Widayanti R, Nugroho HA, Megarani DV, Pakpahan S. 2022. Revealing Spanish mackerel's diversity in Indonesian through local commodities in the fish market. *Biodiversitas* 23 (2): 624-630. DOI: 10.13057/biodiv/d230202.