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RESEARCH ARTICLE

Method Standardization and Guidance Value Determination for Regulation on Formaldehyde in Round Scad (*Decapterus* spp.)

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ABSTRACT

There are reports of traded *Galunggong (Fil.), Decapterus* spp., adulterated with formaldehyde (FA) to lengthen shelf life. FA is a noxious substance with negative repercussions to general consumer health. This warrants the determination of a guidance value due to a lack of a local regulatory value specific to *Galunggong* amidst rising Filipino public concern in the wake of importations complicated by the natural occurrence of FA in the marine fish post-mortem. Comparisons were made on FA levels of fish treated with different conditions exposure and with colorimetric measurements using 405 nm and 412 nm via Nash's method optimized for a fish matrix with no significant difference in measurement to at most 30 ppm FA in an aqueous matrix. There is also no significant difference in FA content of samples measured immediately from those frozen overnight. Therefore, in line with the regulatory mandates of the Bureau of Fisheries and Aquatic Resources (DA-BFAR) of the Department of Agriculture (Republic of the Philippines), a guidance value of 46.24 µg FA/g of *Galunggong* or 46.24 ppm is suggested, which is obtained from FA measurements of fish subjected to minimal anthropogenic treatments in different conditions of adulteration. Samples from different Metro Manila wet markets serve as the baseline. The guidance value is suggested to be used as a reference for regulatory purposes and can be improved with a more widespread sampling of fish from landing to selling.

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1. INTRODUCTION

The Philippines is an archipelago rich with aquatic resources, with its coastal communities relying heavily on fish for protein. Logistics to inland markets require preservation like cooling. *Decapterus* spp., known locally and officially as "Round Scad" or *"Galunggong,"* is a pelagic fish popular for being low-cost and highly available hence monikered as the masses' food with the colloquial name "GG." From the last decade, up until 2016 and 2018, there was a decrease in GG capture, hinting at declining populations (PSA 2011; PSA 2019). Since 2015, fishing seasons were imposed, dwindling local supply and subsequently increasing GG prices. Importations were made to curve this but, in turn, made formaldehyde (FA) adulteration a public concern arising from reports of fish being traded in markets in South (Wahed et al. 2016; Times of India 2020) and Southeast Asia (Tin 2019) being adulterated with FA (Uddin et al. 2014; Sanyal et al. 2017) mainly by dipping in various concentrations of formaldehyde solution.

FA naturally occurs upon organic matter combustion (Kaden et al. 2010; Salthammer et al. 2010). It is a popular preservative to biological specimens (Fox et al. 1985), thus the association to embalming. FA as a noxious substance (USNRCCT 1980) affects mammals with irritation of the mucous membrane upon chronic exposure or lesions in severe cases (Kerns et al. 1983), mild skin irritation (Maibach 1983), and nervous system damage (Songur et al. 2010). FA is known in epidemiological studies (Ulm 1989) upon great exposure (Kaden et al. 2010), a potential human carcinogen affecting the respiratory (Blair et al. 1990), gastrointestinal (Soffritti et al. 1989), and bone marrow (Zhang et al. 2010) cells.

FA is used in fish to preserve its apparent freshness (Bhowmik et al. 2017), sparring the product from lower prices. FA presence is not an immediate indicator of adulteration as it occurs naturally in fruits, meats, and fish (Chung and Chan 2009; Jaman et al. 2015; Wahed et al. 2016) and for marine fish, like GG, is commonly formed at various levels from osmoregulatory salts reducing in fish cells post mortem (Castell et al. 1970; Sotelo et al. 1995; Benjakul et al. 2004; Chung and Chan 2009: Cheng et al. 2015) as described in Figure 1. This reaction is catalyzed by cold storage (Leelapongwattana et al. 2005), further degrading meat quality (Yasui and Lim 1987; Sotelo et al. 1995; Summers et al. 2017). Some species have already elevated FA levels like *Harpodon nehereus*, or *Bombay duck* (Eng.), among other members of the genus *Saurida* (Lizardfishes), under family Synodontidae (Chung and Chan 2009) and Gadidae (Amano and Yamada 1964).

The concern is the anthropogenic adulteration of FA in fish. The ambiguity of preparation and FA ingredient grade are causes of concern. Although there are regulatory limits placed for FA in fish in general in other countries, these values are on an ad hoc basis like in Sri Lanka, which has in place a value of 5.0 mg/kg (ppm) (Democratic Socialist Republic of Sri Lanka 2010) and in India with 4.0 mg/kg for freshwater fish and 100.0 mg/kg (100.0 ppm) for marine fish (FSSAI 2019). The Philippines has yet to develop its regulatory value. The analysis is done by Nash's method (Castell and Smith 1973) via the Hantzsch pyridine synthesis of a photoactive chromophore (Compton and Purdy 1980).

Considering its regulatory mandate and actions, DA-BFAR requires a relatively affordable method, has a short turn-around time, and high sample capacity. Therefore, this study aims to determine the differences in using various wavelengths in the colorimetric analysis of FA in fish and the difference between using a relatively less expensive method and the standard, albeit more expensive. The study also aims to determine a guidance value for regulatory purposes.

2. MATERIALS AND METHODS

The study is comparative in nature. Different samples from different sources like markets and landing sites were analyzed as a baseline. The FA content of fish treated with varying conditions of adulteration was compared to the untreated controls. The matrix effect was also scrutinized. The FA derivative diacetyl-dihydrolutidine (DDH) (Nash 1952) maximally absorbs at λ=412 nm but was measured at λ=405 nm, so comparisons were made in the measurements between the two wavelengths.

2.1 Chemicals and reagents

Deionized distilled water (DDW), used in the subsequent preparations and dilutions, was obtained from a Milli-Q water system (Millipore, MA, USA). The FA standards and simulating solutions diluted in DDW were prepared from an AR grade 37% w/w (3.7 x 105 ppm) solution of formaldehyde (stabilized in 10% methanol) purchased from Scharlau (Barcelona, Spain). For the sample extractions, 5% w/w Trichloroacetic acid (TCA) solution was prepared from dissolved moist crystals of AR grade TCA purchased from Merck KGaA (Darmstadt, Germany) in DDW. Nash's reagent was prepared in DDW with the following final concentrations of 0.003% v/v of A.C.S. reagent grade glacial acetic acid purchased from J.T. Baker (Phillipsburg, NJ, USA), 15.00% w/v of AR grade Ammonium acetate hygroscopic crystals purchased from Himedia (Kennett Square, PA, USA), and 0.002% v/v of AR grade 99.5% Acetyl acetone solvent purchase from Loba Chemie PVT (Mumbai, India).

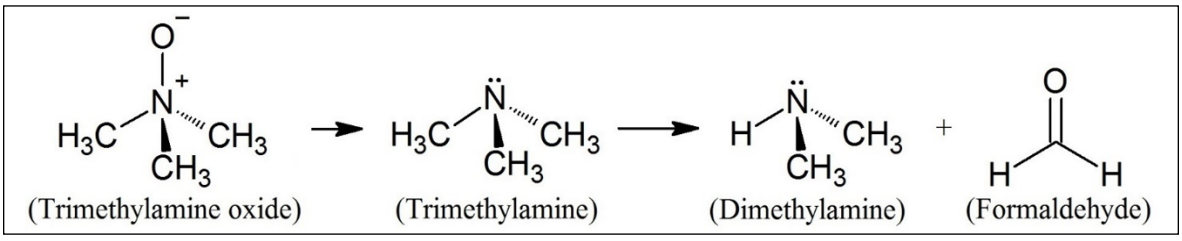

Figure 1. The degradation of Trimethylamine oxide to form volatile bases and Formaldehyde (Chung and Chan 2009)

2.2 Instruments

Instruments and wares used are the following: BioHomogenizer®, hand-held homogenizer manufactured by ESGE Switzerland, Lab. Centrifuge® by Digisystem Laboratory Instrument Inc. BioTek Instruments Inc. (Winooski, VT, USA). Plate reader with model no. ELx800 carried out measurements using the 96-well plate format designated in this study as "PR" and the Shimadzu Asia-Pacific (Singapore) model no. UV-1800 Spectrophotometer carried out measurements using a quartz cuvette designated as "SPM."

2.3 Study area and baseline data

On August 24, 2018, in conjunction with the monitoring order of DA-BFAR, samples of GG were obtained from Farmer's Market (14.62° N, 121.05° E) and Balintawak market (14.66° N, 121.00° E) in Quezon City and Navotas Fish Port (14.64° N, 120.95° E) in Navotas City. Subsequently, Muñoz Market (14.65° N, 121.01° E) in Quezon City was sampled monthly for 2019. A total of four sampling sites were identified, with three sites sampled once and one site sampled monthly. The sites were selected considering popularity, proximity to areas of high population hence high patronage, large output, and proximity from the laboratory for practical reasons. Quezon City and Navotas City are part of the populous Metro Manila. Navotas Fish port is the largest and primary fish landing site of Metro Manila. The state of the GG in the stalls was displayed in the open air but surrounded by tubed ice. To the best of the sampler's inquiry, all the GG samples were landed the day prior, transported under ice, and displayed on the morning of the sampling day. From the selected stalls that display fresh GG with tubed ice, the stalls to be sampled were selected based on price and ease of access. All stalls sampled obtained their fish from Navotas fish port. Most GG landed in the harbor are caught from the West to the southwest seaboard of Luzon, Philippines. The sampling of the stalls is done to obtain a baseline value and evaluate the applicability of the suggested guidance value. The samples obtained here were only analyzed at 405 nm using the plate reader.

2.4 Sample preparation and analysis

A fresh fish batch from a selected stall was set and evaluated by weighing the fish to know if each fish in the batch could provide a representative 5 g of fish flesh. The fish were grabbed blindly from the qualified batch of multiple fish in a bag until five (n=5) fish were obtained. The fish were then fileted on both sides. The resulting two filets were combined, minced, and mixed on a chopping board with a kitchen knife until the sample was pureed into a clay-like solid of homogeneous consistency. The resulting puree was coned and quartered until 5 g was weighed into a 50 mL centrifuge tube. The sample was added with 20 mL of TCA and homogenized with a hand-held homogenizer at the highest setting for 15 seconds. The resulting homogenate was left to stand for 30 minutes at room temperature. The homogenate was then placed at a centrifuge set at 3000 RPM for 10 minutes. The resulting supernatant was decanted and filtered with a Whatman No. 41 filter paper into a 100 mL volumetric flask and diluted to mark with deionized distilled water. The precipitate pellet was disposed of. A 3 mL aliquot was obtained from the diluted sample solution into a capped test tube. Three (3) mL of Nash's reagent was added. The resulting solution was then heated for 15 minutes at 60° C. The sample was then cooled and set for analysis. For the analysis using PR, the 96-well plate was added with the standards and samples. Each sample is loaded into the plate at five trials (t=5), with one well per trial, and analyzed at 405 nm. For the analysis using an SPM, measurements were done with a quartz cuvette and with the 405 nm and 412 nm beam at four trials (t=4) each.

2.5 Standards preparation and analysis

A 1000 ppm FA stock solution is prepared by transferring 0.270 mL of 37% AR grade FA into a 100 mL volumetric flask and diluted to mark. The stock solution is used to prepare 0 ppm, 3 ppm, 6 ppm, 12 ppm, 24 ppm, and 30 ppm standard solutions. Three (3) mL aliquots were obtained from the standard solutions and were added with 3 mL Nash's reagent with the resulting solution heated like the treatment on the samples and subsequently measured alongside the samples.

2.6 Matrix effect and recovery tests

The matrix effect is the "effect of the matrix" on the analysis of the analyte, which may cause further positive or negative deviations from the true value. This effect is evaluated by measurements of background and spiked samples at different dilutions (dilution factor (DF) of 1, 5, 10, and 20), and in this study was done with PR at 405 nm. The obtained

measurements are then compared to each other for any significant differences, the presence of which is indicative of the matrix effect, the evaluation of which is critical in determining the possible interference that must be taken into account during measurement and calculations.

Method validation for this study is somewhat limited since certified reference materials for FA in fish matrix specifically for GG meat are not yet available. CRMs for FA exist but for wastewater or effluent matrices and may not necessarily apply to the method of this study like provided by Supelco® "Formaldehyde in Water" CRM, which is more applicable to chromatographic methods. Instead, on account of costs, availability, and time, the study opted to use the earlier mentioned FA AR grade reagent with a known concentration for spiking. Aqueous solutions of FA at 15 ppm were prepared and measured in PR at 405 nm and SPM at 405 nm and 412 nm. This is done to gauge the applicability of using 405 nm from the standard 412 nm probing wavelength and gauge any differences between using PR versus SPM.

2.7 Comparison of using λ=405 nm vs λ=412 nm and SPM vs. PR

A reagent blank was prepared, denoted with "RB" sample code. Two samples of GG represented as "AG1" and "AG2" were treated with FA by soaking them in a 5% solution for an hour with another separate sample acting as control denoted "G." Two sets of each of the codes were prepared at three trials to be analyzed in the following conditions: the first set was analyzed at PR 405 nm and the second set was analyzed in an SPM at 405 nm and 412 nm, respectively. The different linearity parameters of the calibration curves were also determined by measuring the standards at PR 405 nm, SPM 405 nm, and SPM 412 nm.

2.8 Determination of a recommended guidance value

Samples of GG were selected from a batch purchased in Muñoz Market. First, a fresh fish batch from a selected stall was set and evaluated by weighing the fish to know if each fish in the batch could provide a representative 5 g of fish flesh. Then, the fish were grabbed blindly from the qualified batch of multiple fish in a bag until five (n=5) fish were obtained. Next, the sampled fish were directly soaked in a solution of FA at different concentrations and other different conditions like exposure time and subsequent storage and freezing specified in table 1 to simulate different conditions of adulteration. Finally, the fish were analyzed with the method. Note that the FA solutions were prepared with the assumption that the AR grade solution of FA available in the market with an aqueous concentration of 37% is 100% for convenience. The latter two conditions were also aimed to simulate minimal conditions of FA adulteration. This is done to simulate and obtain the likely FA levels of GG subjected to the minimum of FA adulteration.

2.9 Statistics

Data management is done in Microsoft Excel 2021. Statistical analyses and comparisons of the differences of the conditions and matrix effect were carried out using T-test at α=0.05 and One-way ANOVA at α=0.05 if the comparison is among groups (sample size n=5) with GraphPad Prism 8.0.2 (Free Trial version). The software automatically tests for homoscedasticity upon statistical comparisons. In cases where the variance is equal, comparisons were made with the variance of the mean; otherwise, it resorts to the median, as is the case for large variations. Calibration was done by plotting the measured absorbance of the standards. The resulting plot was then linearly regressed using both MS Excel and Prism to obtain linear parameters used to calculate the samples' concentrations.

3. RESULTS

3.1 Baseline data of formaldehyde in GG

Results for the sample analysis from the three markets and the landing site that would serve as the baseline data were summarized in Figure 2. Measured FA content in the samples in the 2019 series in Figure 2 displayed no significant trend. It was generally consistent throughout the year, suggesting no effect of season changes and time of capture, sale, and sampling.

3.2 Matrix effects and standards spiking

Figure 3 summarizes the data obtained from investigating the "matrix effect" of the GG matrix. The p-values obtained showed insignificant differences of measurement among the dilutions, which suggests insignificant effects of the matrix at the method's analytical concentrations. This supports the use of the aqueous solution of FA without the

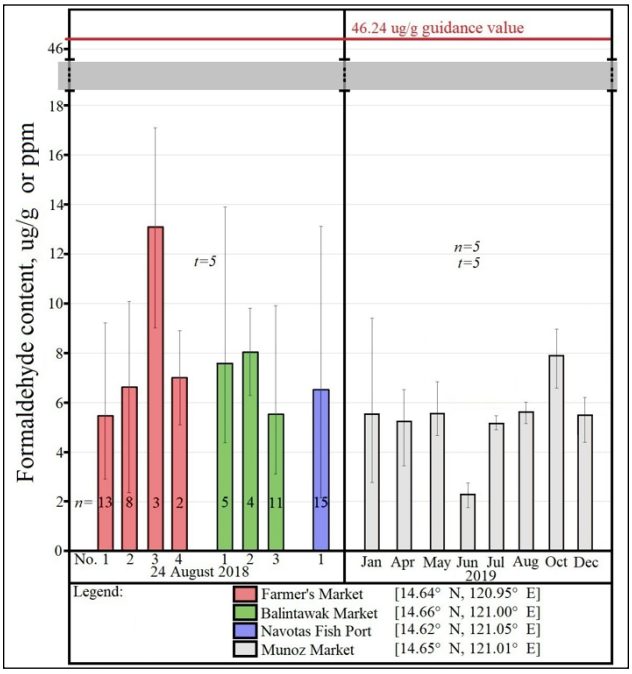

Figure 2. Summary of the measurements of the market monitoring and landing samples which serve as the baseline measured using a Plate reader at 405 nm (PR 405 nm). Seen also is a red line marking the obtained guidance value which is significantly above the obtained background levels of Formaldehyde for reference. Note: *n* is the sample size, *t* is the number of measurement trials per sample.

matrix in standards spiking. As for the measured FA concentrations of the theoretically 15 ppm aqueous FA solutions, there was no significant difference with the measurements carried out using PR at 405 nm and SPM at 405 nm and 412 nm with a p-value of 0.2202 upon comparison with One-way ANOVA, see figure 4. As far as repeatability is concerned, the resulting repeated measurements (n=10) of 15 ppm FA solutions at PR 405 nm resulted in insignificant differences with one another (p=0.1425) (see Figure 4). As for robustness, the difference in the measurements of the 15 ppm solutions using PR 405 nm, SPM 405 nm, and SPM 412 nm was insignificant (p=0.2774) (see Figure 4). As for accuracy, with the prepared standard having a theoretical concentration of 15 ppm, it can be seen in Figure 4 that the mean with the standard deviation of the obtained values encompassed the theoretical value for PR 405 nm, SPM 405 nm, and SPM 412 nm. The limit of detection is calculated for PR 405 nm, SPM 405 nm, and SPM 412 nm is 0.56 ± 0.17 µg/mL, 0.71 ± 0.21 µg/mL and 0.47 ± 0.14 µg/mL, respectively. The limit of quantification is calculated to be 1.68 ± 0.17 µg/mL, 2.15 ± 0.21 µg/mL and 1.42 ± 0.14 µg/mL, respectively.

3.3 Comparisons of the different wavelengths and instruments

Figure 5 summarizes the measured values of the different FA adulteration simulations as mentioned in section 2.8. Figure 5 also summarizes the comparisons of the various conditions under different measurement parameters like wavelength and instrument. Figure 6 summarizes the difference of the generated calibration curves in PR 405 nm, SPM 405 nm, and SPM 412 nm. Said calibration curves are virtually linear as far as the Pearson's linearity coefficient is concerned, suggesting the feasibility of calibration for PR and compliance to the Beer-Lambert relation. As far as differences are concerned, for SPM 405 nm and SPM 412 nm, there were no significant differences in the obtained measurements of the standards between the two different wavelengths. The difference for PR 405 against the two SPM was no longer analyzed as the difference is noticeable.

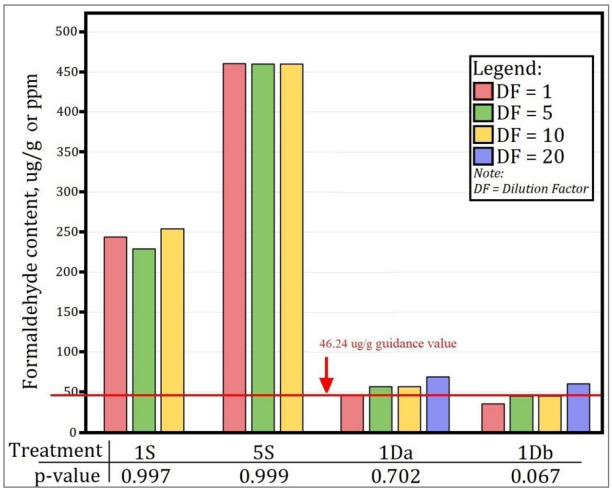

Figure 3. Fomaldehyde levels in µg/g or ppm of samples (from fish subjected to different adulteration conditions) at different dilutions post extraction with p-value from using One-way ANOVA at α=0.05. Note: DF means Dilution Factor. The red line indicates the guidance value obtained. Data obtained is used to evaluate the matrix effect.

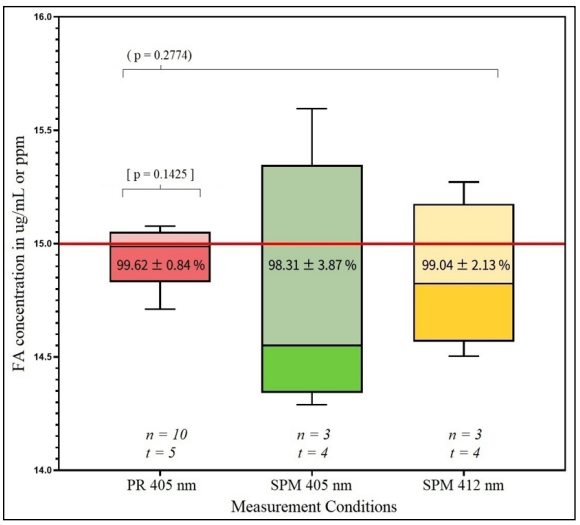

Figure 4. Box graph showing the recoveries of the measurements of 15 ppm Formaldehyde at different measurement conditions. Note: *n* is the sample size, *t* is the number of measurement trials per sample. Shown in the parenthesis is the p-value of the comparison of the means between using Plate reader at 405 nm (PR 405 nm) and using the Spectrophotometer at 405 nm and at 412 nm (SPM 405 nm and SPM 412 nm). Shown in square bracket is the p-value of the comparisons of the means obtained for the Plate reader at 405 nm.

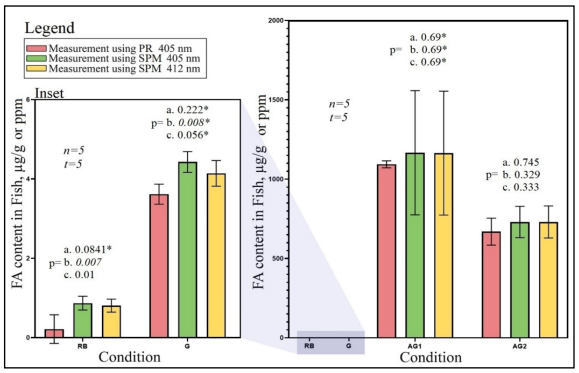

Figure 5. Summary of the Formaldehyde content measured at the two different wavelengths and instruments. Comparinsons of the mean (median* in cases where the mean fails tests for homoscedasticity) of measurement conditions and samples between a. Spectrophotometer at 405 nm (SPM 405 nm) vs. the same instrument at 412 nm (SPM 412 nm), Plate reader at 405 nm (PR 405 nm) vs Spectrophotometer at b. 405 nm (SPM 405 nm) and at c. 412 nm (SPM 412 nm) result in the above p-values. Italicized values indicate significant differences between the two conditions. Note: *n* is the sample size, *t* is the number of measurement trials per sample

3.4 Determination of a guidance value

After analysis of the fish samples subjected to the conditions of FA exposure simulations, conditions 1S and 5S as specified in Table 1 resulted in FA levels that were significantly larger than that of the control, using t=test at α=0.05 with p<0.001 for both conditions. Further, the sample introduced to a higher concentration of FA had higher levels (see Figure 7). Apparent FA levels in conditions 1Da and 1Db are also significantly greater than that of the controls, using t=test at α=0.05, p-values of 0.007 and 0.009, respectively, against the control. There is no significant difference between treatments 1Da and 1Db (p>0.05). The lower limit value obtained from the minimum of FA adulteration that was not subjected to refrigeration is 46.24 µg FA/g or 46.24 ppm (Note: 1 µg/g is equivalent to 1 ppm).

4. DISCUSSION

A guidance value is needed on account of the requirements of the regulatory functions of DA-BFAR and considering the biochemical nuances of FA detection. Methods like Isotope Ratio Mass Spectrometry (IRMS) can be used to detect anthropogenic FA in fish as well as a proposed superiority of using High Performance Liquid Chromatography (HPLC) (Hoque et al. 2018). Another method proposed is comparing the amount of Dimethylamine (DMA) on account of the naturally stoichiometrically equal amounts of the two cleavage products of Trimethylamine oxide (TMAO) even in different conditions. Cases where the amount of FA in

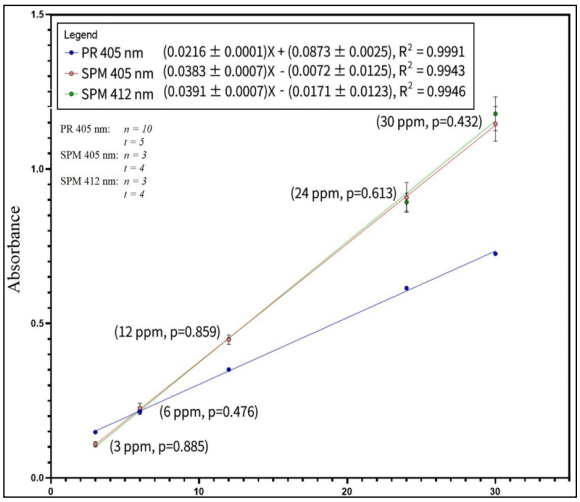

Figure 6. Calibration curves of the measurements using the two different wavelengths and instruments. In parenthesis are the concentrations of the standards and p-values of the comparison between the absorbances measured between 405 nm and 412 nm using the the Spectrophotometer (SPM 405 nm and SPM 412 nm). The calibration curve obtained from measurements using the Plate reader at 405 nm (PR 405 nm) is no longer compared due to the obvious difference. Note: *n* is the sample size, *t* is the number of measurement trials per sample.

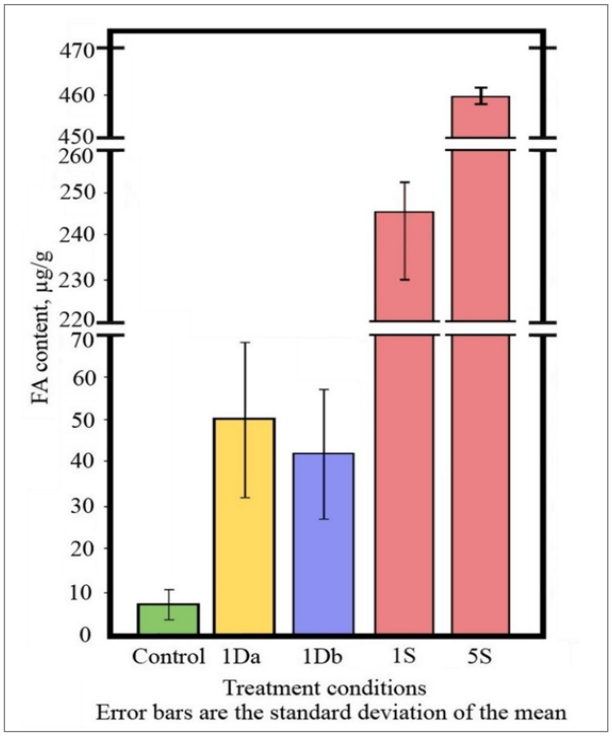

Figure 7. Amount of Formaldehyde in the samples analyzed that were subjected to the simulated conditions of adulteration. Note that the data presented only accounts for the undiluted sample with a dilution factor of 1.

the fish is higher than DMA, indicate adulteration (Shen et al. 2015) with measurements carried out with HPLC (Chung and Chan 2009). However, concerns in the determination of FA are accessibility and affordability, especially for DA-BFAR's central and regional laboratories on the scale of national regulation and the sheer number of samples requiring short turnaround times.A more inexpensive and faster alternative suitable for the DA-BFAR's purposes would be to use spectrophotometric/colorimetric methods to determine anthropogenic FA chemically to a degree rather than chromatographic or nuclear analytical methods. Going further in this vein is the slight preference for PR over SPM in terms of availability, cost, and turnaround time. PR has high sample capacity due to the 96-well plate format, though SPMs can also read in 96-well plate format instead of cuvette format, albeit it tends to be more expensive.

The measured levels of FA in the fish that was adulterated in the laboratory would be significantly elevated statistically compared to the baseline, whose measurement can be attributed solely to the background and naturally produced postmortem levels of FA in the flesh as well as a small amount of matrix interference. A guidance value of 46.24 µg of FA/g fish is obtained from the minimal of the measurements from non-refrigerated adulterated samples, which is value due to the incorporation of the natural background FA levels with the presumed levels from minimal conditions of adulteration; hence any measurement above the mentioned value can and may be grounds for possible adulteration of GG. The US-EPA's reference dose translates to 0.2 mg per kg body weight per day, and the average body weight of Filipino males and females as per the Department of Science and Technology-Food and Nutrition Research Institute's (DOST-FNRI) 8th National Nutrition Survey is at estimated values of 61.3 kg and 54.3 kg, respectively (FNRI-DOST 2013). Considering the data and assuming an ordinary Filipino consumes 100 g of GG for a day, they would have consumed about 0.0745 mg and 0.0852 mg of FA per kg body weight for Filipino males and females, respectively. This suggests that amidst being a value obtained from the sample subjected to presumably the minimum conditions of adulteration, consumption is still deemed safe as far as the reference dose is concerned. In the same vein, this would imply that to reach the threshold that is the reference dose of 0.2 mg per kg body weight per day, one who is 50 kg has to consume for a day 100 g of fish that has an FA content of 100 µg/g fish in which, at least with the presumed parameters, is parallel with the

Table 1. Different conditions and parameters simulating FA adulteration in *Galunggong* (*<1 sec: rapid immersion of the fish being held from the tail, dunked quickly headfirst until fully submerged and immediately removed with excess solution removed by shaking)

Condition	FA (%v/v concentration)	Exposure time (sec or hr)
Control	Control	Control (0 sec)
1S	1%	1 hr
5S	5%	1 hr
1Da	1%	Dipped (<1 sec*)
1Db	1%	Dipped (<1 sec*) + overnight freezing

FA oral exposure limit of the European Food Safety Authority (EFSA) of 100 mg per day (EFSA 2014). The guidance value is below the reference dosage threshold of the US-EPA. However, the concern is the act of adulteration and not immediately the risk of surpassing the threshold since merely adulterating the fish products is a violation under RA 10654 and RA 10611. In addition, the risk posed by using FA in fish is aligned to the risks posed by using AR and lower grades of FA, which may have impurities that are also substances worthy of studying compared to food grade FA if ever it exists. The probability of unprofessional handling and preparation of the reagent and illegal adulteration leading to surpassing the threshold reference dose anyway is not farfetched.

The proposed guidance value is only recommended for *Galunggong* as this is the fish in the study. Due to the considerations of DA-BFAR to use a more available wavelength via relatively less costly PR for the benefit of DA-BFAR's regional laboratories and field activities, 405 nm is the available option for measuring wavelength when conducting colorimetric analysis as opposed to the method's established 412 nm. The measured absorbance of the standards at SPM 405 nm against SPM 412 nm are insignificantly different though it can be observed that the variance increases at higher concentrations. This entails further study on higher concentrations, but for the time being, the insignificant difference is presumed from minimum to at most 30 ppm. Additionally, comparing the slopes of the calibration curves generated from measurements using PR and SPM would be difficult considering the rather large difference between the two instruments (see Figure 6). According to the Beer-Lambert relationship, this could be explained by the fact that path lengths are a factor in the relationship between absorbance and concentration. In short, the path length is related to the slope, which is different between PR and SPM, supported by the fact that PR uses a 96-well plate while SPM utilizes a cuvette. The material of the 96-well plate and the cuvette are also factors. However, differences caused by these factors are compensated with calibration. The same cannot be said about varying the wavelength as this factor changes the molar extinction coefficient in the Beer-Lambert relation. However, concerning 405 nm and 412 nm, the difference is not significantly different at the range of concentration used in this study.

Due to the failure of some means to homoscedastic tests, the medians are used instead with merit because results varied widely among samples (see Figure 5). Representativeness of the sample was primarily limited to the individual fish themselves; this was the rationale of filleting the fish completely, rationalized further by the fact that this is generally the only part of the edible fish, then homogenizing it before the sample was obtained. It is recommended to increase the sample size and expand the parameters on which the fish would be subjected.

It can be recommended to widen the sampling reach and incorporate other fish dealership facilities like fish ports and ambulatory hawkers. Other methods of FA introduction to fish, such as injection, are also recommended to be studied. Additional comparisons and testing for the use of 405 nm in PR against 412 in SPM are also recommended. It is also recommended to conduct a further and detailed risk assessment and profiling considering the Acceptable Daily Intake (ADI) regarding the risks posed by FA adulteration in fish. Other factors like the morphology of the fish, other aspects of the fish's skin, i.e., scale, keratinized layers, thickness, openings, etc., and other physicochemical conditions may come into play that would cause the variation of the FA levels of the fish given the same conditions of exposure to FA; thus, warranting the need of further testing to other species. The species that have naturally elevated levels of FA, as well as other fishes and probable methods of adulteration, have yet to be tested if any other values optimized for these circumstances can be obtained. Factors like pre-consumption treatment of the fish like drying, smoking, and down to cooking like boiling in soup or frying would influence the amount of FA in the fish, which would pose different levels of risk warranting not only additional tests with these factors in mind but also additional points for risk assessments

5. CONCLUSION

The guidance value of 46.24 μg/g obtained from the study would be helpful and will provide for the regulatory functions of DA-BFAR and other regulatory entities. As far as what was covered in the study is concerned, there is a statistically significant difference between presumably unadulterated fish and the fish subjected to the minimal conditions. This finding would be the basis for regulatory actions when analyzing a sample. It can also be concluded that since using 405 nm is not significantly different from using 412 nm if the measured concentrations are at confidently at most 30 ppm and the difference of PR and SPM is remedied with calibration, PR 405 nm is a viable and more apt option for DA-BFAR's functions.

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AUTHOR CONTRIBUTIONS

Dela Cruz J: Conceptualization, Methodology, Software, Writing—reviewing, Writing—Original Draft Creation, Editing, Formal Analysis. **Cudia PBKR:** Visualization, Investigation, Methodology. **Seguerra AMM:** Visualization, Investigation, Data Curation. **Romero MLJ:** Supervision, Writing—reviewing, Editing, Project Administration.

CONFLICT OF INTERESTS

The authors declare that there is no known conflict of interest.

ETHICS STATEMENT

No animal or human studies were carried out by the authors. The study and the authors adhered with the utmost compliance to modern ethical standards. The test fishes are all procured post-mortem and are already available for human consumption. To the best of the authors' knowledge, the authors release themselves from any ethical concerns involving the monger of the fish prior to purchase. On the environment: chemicals, reagents, and wastes are stored and disposed of properly in compliance with environmental regulations. No identifying information of the fishmongers is recorded for and included in the article; therefore, consent is not necessary; only the general location of the sampling site is recorded for reproducibility.

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