

**DETECTION AND QUANTIFICATION OF FORMALDEHYDE CONTENT OF
MARINE FISHES SOLD IN WET MARKET IN SELECTED
CITIES OF NUEVA ECIJA**

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ABSTRACT

VILLACORTA, MICHELLE ANGELA G., Department of Pathobiology, College of Veterinary Science and Medicine, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, **June 2019, DETECTION AND QUANTIFICATION OF FORMALDEHYDE CONTENT OF MARINE FISHES SOLD IN WET MARKET IN SELECTED CITIES OF NUEVA ECIJA**

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The detection and quantification of formaldehyde content of marine fishes sold in wet markets of Nueva Ecija was the focus of this study. Specifically, it compared the quantity of formaldehyde content in fishes sold in the selected cities of the said province.

A total of twenty-seven (27) fish samples from randomly selected local markets in cities of Gapan, San Jose and Cabanatuan were collected. Fish samples were subjected to extraction and quantitative determination of formaldehyde by UV/Vis spectrophotometry.

Results revealed the presence of formaldehyde content in the samples with the mean range of formaldehyde from 0.00004 to 0.040922 ppm. All the samples detected with formaldehyde were way below the permissible limit suggesting that there was no deliberate formalin contamination to the fishes.

Keywords: formaldehyde, marine fish, Nash test, UV/vis spectrophotometry

LITERATURE CITED

- Aini, N., Siddiquee, S. & Kamaruzaman, A. (2016). Development of formaldehyde biosensor for determination of formalin in fish samples; malabar red snapper (*lutjanus malabaricus*) and longtail tuna (*thunnus tonggol*). Biotechnology research institute, sabah, malaysia. Biosensors 2016, 6, 32; doi:10.3390/bios6030032
- Akshath, U. S., Selvakumar, L. S. & Thakur, M. S. (2011). Detection of formaldehyde in food samples by enhanced chemiluminescence. Anal. Methods, 2012, 4, 699. Doi: 10.1039/c2ay05608a.
- Barut, N. C., Santos, M. D., Mijares, L. L., Subade, R., Armada, N. B. & Garces, L. R. (2003). Philippine coastal fisheries situation, p. 885 - 914. In g. Silvestre, I. Garces, i. Stobutzki, m. Ahmed, r.a. valmonte-santos, c. Luna, I. Lachica-aliño, p. Munro, v. Christensen and d. Pauly (eds.) Assessment, management and future directions for coastal fisheries in asian countries. World fish center conference proceedings 67, 1 120 p.
- Begum, M., Newaz, A. W., Dewanl, B. & Kamal M. (2013). Prevalence of formalin contamination and its effect on organoleptic characteristic of giant fresh water prawn (*macrobrachium rosenbergii, de man*) in different markets of mymensingh. Applied science report. 2 (2):32-38)
- Bureau of Agricultural Statistics. (2014). Fisheries statistics of the Philippines 2008-2010. Department of Agriculture. Quezon city. P. 402.
- Bureau of Fisheries and Aquatic Resources. (2001). Philippine fisheries profile. Philippines: Quezon city.
- Bureau of Fisheries and Aquatic resources. (2007). Managing aquaculture and its impacts: a guidebook for local government. Diliman, Quezon city.
- Camacho, A. S. & Macalincag-Lagua, N. (1988). The Philippine aquaculture industry. In J. V. Juario & L. V. Benitez (eds), perspective in aquaculture development in south east Asia and Japan: contribution of the seafdec aquaculture department. Proceedings of the seminar on aquaculture development in Southeast Asia, 8-12 September 1987, Iloilo city, Philippines. P. 91-116. Tigbauan, Iloilo, Philippines: seafdec, aquaculture department.
- Castell, C. H. & Smith, B. (1973). Measurement of formaldehyde in fish muscle using tca extraction and Nash reagent. J. Fish. Res. Board can. 30:91-98.

- Chromedia. (2018). Uv/vis spectrometry basics. Retrieved on January 07, 2018 from the world wide web: <http://www.chromedia.org/chromedia?waxtrapp=lnyabeshiembpdmbijecatbn&caller=pdoxfdshiembpdmbijejajdj%zoekres=yes&scrsv=sublnyabeshiembpdmbijecatbn>
- Central Intelligence Agency. (2007). The world factbook. Retrieved on May 27, 2019 from the world wide web: <http://www.cia.gov/library/publication/the-world-factbook/>.html
- Citizen's charter. (2007). City of San Jose. Retrieved on January 03, 2018 from world wide web: <http://www.sanjosecity-ne.gov.ph/content/doc/arta-april102017>.
- City of Gapan. (2016). History of Gapan city. Retrieved on January 02, 2018 from the world wide web: <http://www.cityofgapan.gov.ph/gapancityofficialwebsite/index.html>
- Cui, X., Fang, G., & Wang, S. (2007). Kinetic spectrophotometric method for rapid determination of the trace formaldehyde in foods. *Anal. Chim. Acta*, 590: 253-259. Doi: 10.1016/j.aca.2007.03.042.
- Definition of Aquaculture. (1988). Seventh session of the Indo-Pacific fisheries council (ipfc) working party of expert on aquaculture, Bangkok, Thailand.
- Environmental Protection Agency – U.S. (2000). Formaldehyde hazard summary profile. Environmental criteria and assessment office, office of health and environmental assessment, office of research and development, Cincinnati, OH.
- Fao. (2007). The state of world aquaculture and fisheries 2006. Food and agriculture organization of the United Nations. Fisheries and aquaculture department. Rome, Italy.
- Fao. (2008). Yearbooks of fisheries statistics. Summary tables. Food and agriculture organization of the United Nations. Fisheries and aquaculture department. Rome, Italy.
- Fao. (2012). The state of world fisheries and aquaculture 2012. Food and agriculture organization of the United Nations. Fisheries and aquaculture department. Rome, Italy.
- Fao. (2014). Food and agriculture organization of the United Nations. The state of world fisheries and aquaculture, opportunities and challenges. Rome. Italy. P. 3-5.

- Fishbase. (2017). *Caesio cuning*. Retrieved on January 03, 2018 from the world wide web: <http://www.fishbase.org/summary/caesio-cuning.html>.
- Fishbase. (2017). *Decapterus macarellus*. Retrieved on January 03, 2018 from the world wide web: <http://www.fishbase.org/summary/993>.
- Fishbase. (2018). *Euthynnus affinis*. Retrieved on October 10, 2018 from the world wide web: <http://www.fishbase.org/summary/speciessummary.php?id=96&at=jodari>.
- Goon, S., Bipasha, M. S. & Islam M. S. (2014). Fish marketing status with formalin treatment in Bangladesh. *Ijphs*. Vol. 3:2. Pp. 95-100. Issn: 2252-8806.
- Islam, R., Mahmud, S., Aziz, A., Sarker, A. & Nasreen, M. (2015). A comparative study of present status of marketing of formalin treated fishes in six districts of Bangladesh. *Food and nutrition sciences*, 6, 124-134. [Http://dx.doi.org/10.4236/fns.2015.61013](http://dx.doi.org/10.4236/fns.2015.61013)
- Jaman, N., Hoque, S., Chakraborty, S. C., Hoq, E. & Seal, H. P. (2015). Determination of formaldehyde content by spectrophotometric method in some fresh water and marine fishes of Bangladesh. *International journal of fisheries and aquatic studies* 2015; 2(6): 94-98
- Joshi, R., Bhatta, R., Paudel, P. N. & Kafle, B. K. (2015). Formaldehyde content of selected fish from the wet markets of Kathmandu valley. *International food research journal* 22(4): 1434-1437.
- Juliano, R. O., Guerrero III, R. & Ronquillo, I. (1989). The introduction of exotic aquatic species in the Philippines, p. 83-90 in: de Silva, S.S. (ed.) *Exotic aquatic organisms in Asia*. Preceding of the workshop on introduction of exotic aquatic organisms in Asia. *Asian fish. Soc. Spec. Publ.* 3, 154 p.
- Jung, S. H., Kim, J. W., Jeon, I. G. & Lee, Y. H. (2001). Formaldehyde residues in formalin-treated olive flounder (*paralichthys olivaceus*), black rockfish (*Sebastes schlegeli*) and seawater. *Aquaculture*, 194:253-262. Doi: 10.1016/S0044-8486(00)000530-5.
- Nash, T. (1953). The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochemical j.* 55:416-21. Air hygiene lab., public health laboratory service, London, England.

- National Statistics Office. (1990). Province profile: Nueva Ecija. Manila, Philippines. P. 1-7.
- Noordiana, N., Fatimah, A. B. & Farhana Y. C. (2011). Formaldehyde content and quality characteristics of selected fish and seafood from wet markets. *International food research journal*. 18: 125-136.
- Osha, (1999). Formaldehyde and its hazard effects in human. Retrieved on January 18, 2018 from world wide web : <http://www.osha.gov/sljc/formaldehyde/hazards.html>.
- Philippines cities. (2010). History of Cabanatuan. Retrieved on January 19, 2018 from world wide web : <http://philippinescities.com/cabanatuan-city-nueva-ecija/>
- Philippine Information Agency. (2017). Province Nueva Ecija. Republic of the Philippines.
- Philippine primer. (2017). Expat's guide to common fishes in the Philippines. Retrieved on January 02, 2018 from the world wide web: <http://www.primmer.com.ph/tips-guide/2017/08/13/expats-guide-to-common-fishes-in-the-philippines/>
- Samson, E. (1984). The Milkfish industry in the Philippines. In J. V. Juario, R. P. Ferraris, & L. V. Benitez (Eds.), *Advances in milkfish biology and culture: Proceedings of the Second International Milkfish Aquaculture Conference*, 4-8 October 1983, Iloilo City, Philippines. (pp. 215-228). Metro Manila, Philippines: Published by Island Pub. House in association with the Aquaculture Department, Southeast Asian Fisheries Development Center and the International Development Research Centre.
- Sanda, F. M., Victor, M., Toderaş, E., Monica, A., Cărăban, A. (2012). Spectrophotometric measurements techniques for fermentation process (part one) base theory for uv-vis spectrophotometric measurements internal report. University of Oradea, Romania
- Sotelo, C. G., Piñeiro, C. & Pérez-Martín, R. I. (1995). Denaturation of fish protein during frozen storage: role of formaldehyde. *Zeitschrift für lebensmittel-untersuchung und forschung*, 200:14-23
- Tashkov, W. (1996). Determination of formaldehyde in foods, biological media and technological materials by head space gas chromatography. *Chromatographia*, 43(11/12):625-627.

da, M., Frank, N., Sato, S., & Sugimura, T. (1988). Marked increase in the urine level of n-nitrosothioprolin after ingestion of cod with vegetables. *Cancer research*, 48:4049–4052.

lin, R., Wahid, M. I., Jesmeen, T., Huda N. H. & Sutradhar, K. B. (2011). Detection of formalin in fish samples collected from Dhaka city, Bangladesh. *S. J. Pharm Sci*. 4(1): 49-52.

ng, S., Cui, X. J. & Fang, G. (2007). Rapid determination of formaldehyde and sulfur dioxide in food products and Chinese herbals. *Food chemistry* 103: 1487–1493.

ng, S., Cui, X. J., Fang, G., Yasuhara, A. & Shibamoto, T. (1995). Rapid determination of formaldehyde and sulfur dioxide in food products and Chinese herbals quantitative analysis of volatile aldehydes formed from various kinds of fish flesh during heat treatment. *Journal of agricultural and food chemistry* 43:94–97.

What is Schiff's reagent. (2005). Retrieved on January 04, 2018 from the world wide web: <http://stainsfile.info/stainsfile/stain/schiff/schiffwhatis.htm>.

Worldfish center. (2002). Fish: an issue for everyone. A concept paper for fish for all.

WHO. (1989). Environmental health criteria for formaldehyde. Volume 89. World health organization, Geneva, Switzerland.