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Establishment of sensory threshold levels of geosmin and 2-methylisoborneol for Filipinos

[1]Jade G. Pahila, [1]Encarnacion Emilia S. Yap, [2]Rex Ferdinand M. Traifalgar, [2]Crispino A. Saclauso

[1] Institute of Fish Processing Technology, University of the Philippines Visayas, Miagao 5023 Iloilo, Philippines; [2] Institute of Aquaculture, University of the Philippines Visayas, Miagao 5023 Iloilo, Philippines. Corresponding author: E. E. S. Yap, emiliasyap@yahoo.com

Abstract. A study on the specific sensorial threshold levels of two common off-flavour compounds in water systems (geosmin and 2-methylisoborneol) was done through a series of sensory evaluation tests. Two different threshold levels were determined namely: the absolute or detection threshold level and the terminal or saturation threshold level, individually for both geosmin and MIB in aqueous solutions. Results of this study present specific threshold levels for each compound. In addition, using the pooled results of collective sensory responses of selected panellists, it was observed that the detection and terminal threshold levels of 2-methylisoborneol were relatively lower compared to that of geosmin. Geosmin had an absolute (detection) threshold level in an aqueous solution of 18 ng L^{-1} and a terminal (saturation) threshold level in an aqueous solution of 1700 ng L^{-1}, while 2-methylisoborneol had absolute and terminal threshold levels at 14 ng L^{-1} and 900 ng L^{-1} respectively. A psychophysics law known as the Webner-Fechner model was correlated with the results of the sensory tests and results revealed that the sensory intensity perception responses of the panellists followed a logarithmic function in relation to off-flavor compound concentration as expressed in the equation: Intensity = m log (concentration) + b.

Key Words: detection level, saturation level, geosmin, 2-methylisoborneol, off-flavour compounds.

Introduction. Off-flavors in waters are caused by the presence of the compounds geosmin and 2-methylisoborneol (MIB), which are produced by several algal and bacterial species during eutrophication processes (Schrader & Summerfelt 2010; Tanaka et al 1996). In particular, the compound geosmin is reportedly produced by some species of actinomycetes, *Nocardia cummidelens, Nocardia fluminea*, *Streptomyces luridiscabiei*, and *Streptomyces cf. albidoflavus*, that were isolated from a recirculating aquaculture system (Schrader & Summerfelt 2010). Some species of cyanobacteria were also found to produce both geosmin and MIB as well (Izaguirre et al 1982; Tabachek & Yurkowski 1976).

Although the presence of these compounds in water systems, and other associated crops, imparts undesirable odours and tastes among consumers it has not been designated by the United States Environmental Protection Agency and the World Health Organization as hazardous compounds that could affect human health (Young et al 1996). Other studies have also shown that both compounds are neither mutagenic nor cytotoxic (Dionigi et al 1993). This just implies that the major concern with regards to the presence of geosmin and MIB in water is the overall acceptability of this resource and the potential reduction of marketability of commodities such as fish that are reared in water-based systems (Pahila & Yap 2013).

Trans-1,10-dimethyl-trans-9-decalol, or commonly known as geosmin is a secondary metabolite compound responsible for the earthy flavour of drinking waters, which is often branded as undesirable for drinking by most consumers. Although relatively non-toxic, the odour imparted by this volatile compound is generally deemed unacceptable by many consumers. The sensory detection limits for geosmin varies, according to several studies as shown in Table 1.

Table 1

Different threshold limits of geosmin and MIB in water

Geosmin		*MIB*	
Concentration (ng L^{-1})	References	Concentration (ng L^{-1})	References
200	Safferman et al (1967)	18-20	Persson & York (1978)
130	Lillard & Powers(1975)	20	Van Gemert & Nettenbreijer (1977)
50	Medsker et al (1969)	29	Persson and York (1978)
21	Buttery & Garibaldi (1976)	35	Howgate (2004)
15	Van Gemert & Nettenbreijer (1977); Howgate (2004)	100	Medsker et al (1969); Wood & Snoeyink (1977); Rosen et al (1970)
4	Young & Suffet (1999)	4.9	Urase & Sasaki (2013)
3.2	Zhang et al (2006)	4	Rashash et al (1996)
10	Rashash et al (1996)	9	Jensen et al 1999
1	Tempere et al (2011)		

The compound 2-methylisoborneol or 1,2,7,7-tetramethyl-exo-bicyclo [2.2.1]-heptan-2-ol (MIB) has been extensively studied (Medsker et al 1969; Gerber 1965; Rosen et al 1970; Izaguirre et al 1982) and it has been shown that this compound is a natural metabolite produced by Actinomycetes in water (Juttner & Watson 2007). It has a very low detection threshold level which ranges from 4 ng L^{-1} to 100 ng L^{-1}, which varies from different studies (Table 1). At higher concentrations, MIB has been noted to give off an odour similar to camphor (Gerber 1969). This compound is accountable for the “musty” flavour in fresh water systems, such as water reservoirs and aquaculture systems.

Sensory evaluation analysis is done to make use of human panellists as measuring instruments, and to eliminate all possible biases by making use of the best existing techniques developed (Meilgaard et al 2006). In relation to sensory perception, one of the most important characteristics of a compound for it to be perceived is its potency which refers to the amount of the compound necessary to evoke a response from an individual. The potency of a particular compound is roughly defined by the compound’s minimum detectable threshold (Howgate 2004).

The limits of sensory capacities called threshold, defined by Meilgaard et al (2006), could either be: the absolute threshold which is the minimum level of stimulus that can be perceived; the recognition threshold which is the level of a specific stimulus at which it can be detected or recognized; the difference threshold which is the degree of changes in the level of stimulus at which it can be differentiated; and the terminal threshold which is the maximum level of a stimulus where difference in increasing levels can no longer be perceived and often accompanied by pain or discomfort stimulus. Howgate (2004) simplifies this concept as “the lowest concentration of a compound in a medium that can be detected”. According to Laing (1987), the threshold is a value on a stimulus continuum, and not a fixed point, where it is described that a person’s specific threshold is not the detection of a particular stimulus at X% of significance, but rather the concentration he can detect “50% of the time”.

Despite the concise definitions for sensory thresholds, no widely recognized standard procedure for detecting sensory thresholds is considered. Numerous experimental protocols for defining thresholds have been published but these generally differ depending on the type of data collected, the way samples are presented to panellists, and the formulas for calculating thresholds (Howgate 2004).

In the case of threshold determination for geosmin and MIB, numerous studies are present in literature presenting a wide range of detection thresholds. The wide range in threshold limits reported in these studies is attributed to differences in experimental procedures, materials used, and the criterion for defining thresholds (Howgate 2004).

To date, there have been minimal studies on threshold limits of off-flavors in fishery products among Asian consumers and Filipinos in particular. Hence, this study aimed to establish two different threshold levels for both geosmin and MIB: [1] the absolute threshold (detection threshold), defined as the minimum concentration of the stimulus or the compound that is capable of producing a sensation or detection; [2] the terminal threshold (saturation threshold), defined as the extent of a stimulus or compound concentration where there is no increase in intensity in the perception of the panellists (Meilgaard et al 2006). Establishing such initial threshold limits for geosmin and MIB in the Filipino context will therefore provide valuable information for efforts in managing off-flavor compounds in fishery products.

Material and Method. Sensory threshold limits for geosmin and MIB were established using data from sensory perception of Filipino panellists. Sensory evaluation tests were conducted at the Institute of Fish Processing Technology Sensory Evaluation Laboratory of the University of the Philippines Visayas from January to March 2014.

Materials and preparation. Separate aqueous solutions of geosmin and MIB with varying concentrations were prepared from analytical standards (*Supelco® Analytical*) which initially contained 100 µg mL^{-1} of each compound in methanol. Triple distilled water was used for the series of dilutions. Air tight sealed scintillation vials were used to hold the prepared solutions.

Sensory threshold determination test. Different concentrations of geosmin and MIB were prepared individually from the analytical standard solution and triple distilled water. Geosmin concentrations started from 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 40, 50, 100, 200, 400, 800, 1600, 1700, 1800, 2000, to 2200 ng L^{-1} while MIB concentrations were prepared at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, to 2000 ng L^{-1}. Prepared solutions were evaluated by the panellists according to the intensity perceived, which was done in three independent runs to ensure precision of results. A 15 centimetre intensity line scale, with anchors at both ends that correspond to “no detection” at the leftmost and “very strong detection” at the right most, was used for the evaluation of intensity of each sample solution as suggested by Cox et al (2001) similar to the recommendation of Yeh et al (1998). Sensory scorecard used for this evaluation is reflected in the Annex 1. This form of scoring allows the recognition of greater differentiation in a non-parametric analysis. The tests were divided into batches with maximum of 6 samples each, to avoid sensorial/olfactory saturation and stress to the panellists. Panellists were selected based on their background and experience on sensory evaluation or preferably those who have sufficient theoretical background and experience in sensory evaluation.

Data analysis. Responses were measured and analysed as continuous parametric data, and sensory responses which were detected 50% of the time were used as basis of the establishment of threshold levels (Anderson 1970; Meilgaard et al 2006; Stone et al 2012). A similar basis was used by Howgate (2004) in a review on the subject of the uptake and degradation of geosmin and MIB.

Results and Discussion. Individual sensory intensity responses of each panellist for a particular compound concentration were measured. Measured sensory responses for lower concentrations were used for the analysis of the absolute threshold and sensory responses for higher concentrations at the end of the concentration range were used for the analysis of the terminal threshold.

Absolute (detection) threshold for geosmin and MIB. For the determination of the absolute threshold limit of the panellists for a specific compound, the objective of this part of the study was to identify the lowest concentration of the compound prepared in a solution that is able to elicit a positive detection response among the panellists for more than 50% of the time.

For MIB, concentrations from 0 to 12 ng L^{-1} had response scores of zero or “no odour perceived” (Figure 1), with a frequency count of more than 50% of the total responses. The first concentration which had more than 50% of panellists giving a response score higher than zero (mean = 0.89) was at 14 ng L^{-1}. The absolute threshold for MIB was therefore pegged at 14 ng L^{-1} since more than 50% of the panellists were able to detect and perceive the particular odour of MIB at this concentration.

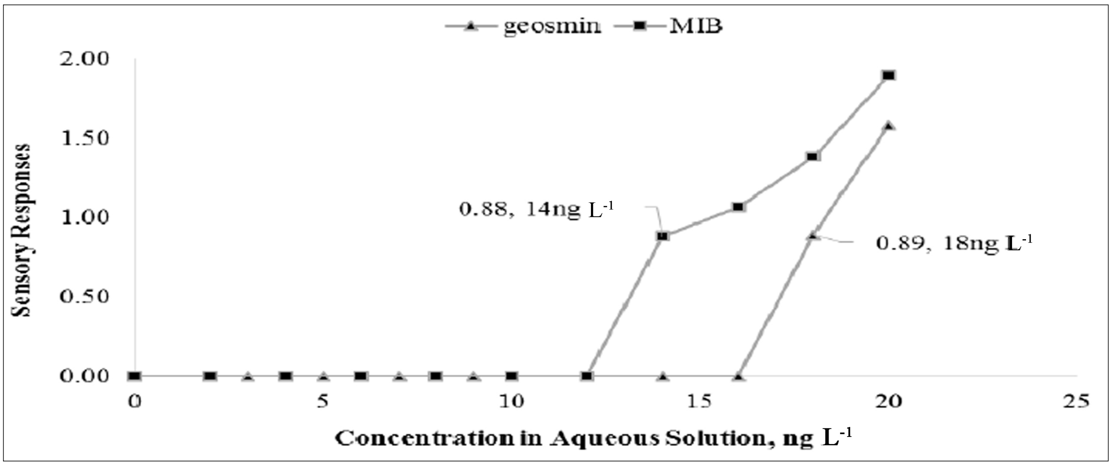

Figure 1. Absolute (detection) threshold for geosmin and MIB expressed as concentration (ng L^{-1}) vs. mean sensory responses (n = 27).

***Terminal threshold for geosmin and MIB**.* Since the terminal threshold is the saturation point of the panellists where no further perceivable increase in sensorial intensity can be detected as the concentration of the specific stimulus increases (Meilgaard et al 2006), the objective for this part of the study was to identify the minimum concentration of geosmin and MIB when the panellists are unable to discriminate changes in perceived intensity with increasing concentration of a particular compound.

The means of all 27 sensory responses for the different concentrations of geosmin solutions from 400 ng L^{-1} to 2200 ng L^{-1}, and 600 ng L^{-1} to 2000 ng L^{-1} for MIB (Figure 2) show sensory responses tapering off at the higher concentrations starting from 1700 ng L^{-1} for geosmin and 900 ng L^{-1} for MIB. The point at which responses start to show no significant difference between concentrations corresponds to the value of the terminal threshold or the saturation point as established by the representative panellists. Based on the line scale used in the sensory evaluation, a score of 15 indicates a "very strong detection", where no recognizable increase in concentration is perceived. It is at concentrations of 1700 ng L^{-1} for geosmin and 900 ng L^{-1} for MIB that a sensory response score of 15 was given by more than 50% of the total respondents. Therefore the terminal threshold values for geosmin and MIB were pegged at 1700 ng L^{-1} and 900 ng L^{-1} respectively.

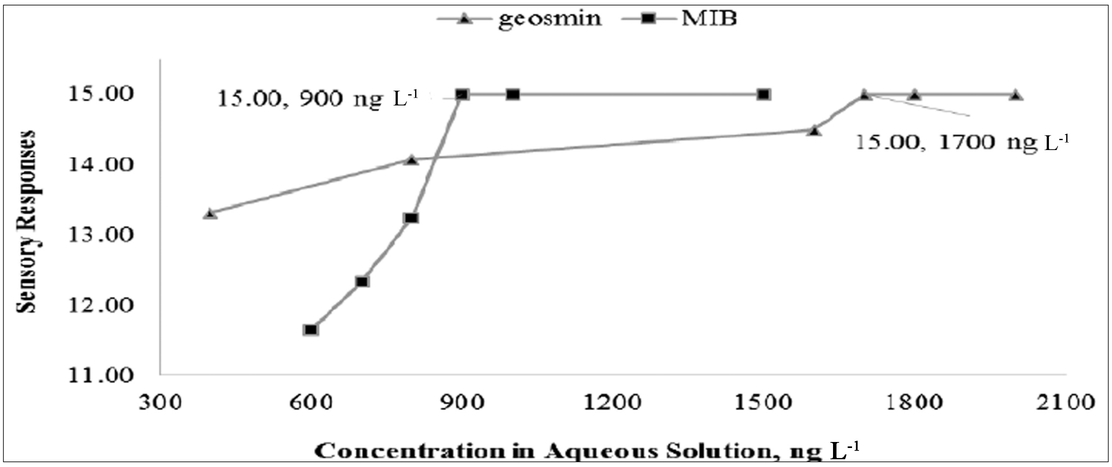

Figure 2. Terminal threshold for geosmin and MIB expressed as concentration (ng L^{-1}) vs. mean responses (n = 27).

Cross cultural differences in sensory perception. The results of these tests presented specific absolute and terminal threshold levels for geosmin and MIB compounds for Filipino panellists with an age range from 20 to 21 years old. In comparison with some published data on the threshold levels of geosmin (Table 1) ranging from 4-200 ng L^{-1}, results showed that the particular group of Filipino panellists who participated in this study had relatively lower absolute threshold levels at around 18 ng L^{-1}. Similarly, threshold levels for MIB at around 14 ng L^{-1} in this study were also comparatively at the lower range of values compared to published absolute thresholds (Table 1) ranging from 4 to 100 ng L^{-1}.

Some cross cultural studies of Ferdenzi et al (2011), Hong et al (2011), Ayabe-Kanamura et al (1998), Prescott (1998), and Yeh et al (1998) on the differences in sensory perceptions pointed out that individual experiences of the panellists, behavioural differences in culture, and personal preferences influence the difference in sensory perception. Prescott & Bell (1995) pointed out in a review on cross cultural determinants of food acceptability that results of a sensory perception study done by a different culture may not be applicable to other cultures when used. This could therefore explain the differences in the sensory perceptions of the different panellists on geosmin and MIB, in comparison to other similar published studies. This therefore puts great significance on the current study, that it is based on extensive literature research, the first documentation of sensory perception thresholds for geosmin and MIB among Filipino panellists.

Webner-Fechner model of the sensory responses with varying concentrations of geosmin and MIB. In the study of Whelton & Dietrich (2004) on the relationship of concentration and perception of some water odorants, a Webner-Fechner model was used in plotting the values of particular odorants versus its perceived intensity. This model is mainly used in psychophysics wherein the Webner model describes the linear relationship of the intensity of perception to a certain stimuli concentration while the Fechner model further postulates Webner's law stating that the external stimulus is scaled into a logarithmic representation of intensity of perception (Dehaene 2003). It can also simply be described that the intensity of perception is a logarithmic function of stimuli concentration using the equation: Intensity = m log (concentration) + b (Whelton & Dietrich 2004).

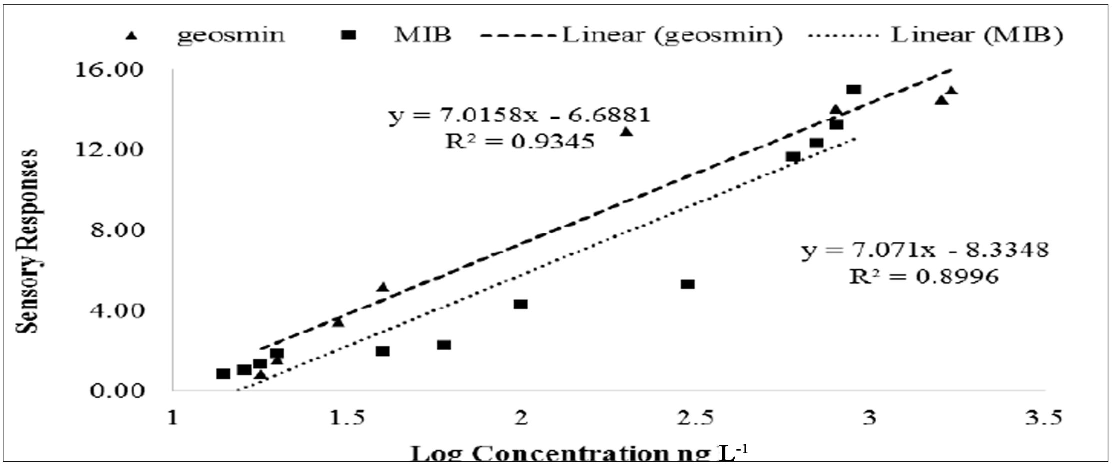

Figure 3. Webner-Fechner plot of the different concentrations of geosmin and MIB in aqueous solution between the absolute (detection) threshold and the terminal threshold.

Using the Webner-Fechner model, values obtained from the threshold test were plotted in a scatter plane as the logarithmic concentration of each compound versus the sensory responses (Figure3). From this, linear regression analysis was performed to obtain linear equations to describe each of the compounds tested. Values plotted were those at discriminable concentrations, or those which fall in between the absolute threshold and the terminal threshold since these were values at the given concentrations that can be detected by the panellists but not up to saturation.

Based on the graph in Figure 3, the plot of the sensory responses versus the log of concentration (ng L^{-1}), geosmin had a linear equation of y = 7.0158x - 6.6881 and a coefficient of determination of R^2 = 0.9345 using 8 points from 18 ng L^{-1} (the absolute threshold) up to 1700 ng L^{-1} (the start of the terminal threshold). The values obtained by MIB on the same plot had a linear equation of y = 7.071x - 8.3348 and a coefficient of determination R^2 = 0.8996. It can be observed from the plot that the MIB responses were lower than that of geosmin, and both series are observed to have a nearly constant parallel pattern. This was similar to observations by Whelton & Dietrich (2004), wherein odour intensities for geosmin and MIB had slight variations indicating different perception levels even for very similar compounds. Given these linear equations for geosmin and MIB, a particular sensory response for a specific concentration of the compound which coincides within the values of the given curve may be predicted for this particular set of panellists.

Conclusions. This study has established the first reported threshold levels for the off-flavor compounds geosmin and MIB, which is specific for Filipino panellists. The minimum concentration of geosmin and MIB in water that could be detected and perceived by the set of panellists were at concentrations of 18 ng L^{-1} and 14 ng L^{-1} respectively. Below these concentrations, majority of the panellists were unable to detect the presence of the compounds in water. The minimum concentration of geosmin and MIB in water that can cause saturation to the olfactory senses of the panellists were at concentrations 1700 ng L^{-1} and 900 ng L^{-1} respectively, and beyond these concentrations panellists were unable to discriminate the effects of the increasing concentration of the compounds. The Webner-Fechner model of the sensory responses in relation to the logarithmic concentration of the compounds is an illustration of a possible estimation of the response of the panellist at a given concentration within the scope of the given equation as a standard curve which are, for this instance, y = 7.0158x - 6.6881 for geosmin and y = 7.071x - 8.3348 for MIB.

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Authors:
Jade G. Pahila, Institute of Fish Processing Technology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, 5023 Miagao, Iloilo, Philippines, e-mail: jade_go_pahila@yahoo.com
Encarnacion Emilia S. Yap, Institute of Fish Processing Technology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, 5023 Miagao, Iloilo, Philippines, e-mail: emiliasyap@yahoo.com
Rex Ferdinand M. Traifalgar, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, 5023 Miagao, Iloilo, Philippines, e-mail: skerferd@yahoo.com
Crispino A. Saclauso, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, 5023 Miagao, Iloilo, Philippines, e-mail: casaclauso@yahoo.com

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ANNEX

Sensory Detection Threshold Score Card

Name: ___________________________________ **Age:** ____ **Sex:** ___ **Date:** __________

Instructions: Coded samples are presented. Open one sample one at a time, gently sniff and evaluate accordingly. Please place a mark on the scale provided corresponding to the intensity perceived.

Example1:
Sample
840

No detection — Very strong detection

If no odour is perceived please encircle the line scale anchor corresponding to "no detection".

Example2:
Sample
589

No detection — Very strong detection

If odour perceived is too strong to categorize intensity, please encircle the line scale anchor corresponding to "very strong detection".

Example3:
Sample
396

No detection — Very strong detection

Please take at least two minute break in between samples.

Sample Code

______ No detection — Very strong detection

______ No detection — Very strong detection

______ No detection — Very strong detection

______ No detection — Very strong detection

______ No detection — Very strong detection

______ No detection — Very strong detection

Comments:
____
____

Thank you!

**This scorecard presented is scaled down to fit printable page size.*