

## Reduction of off-flavour compounds (geosmin and 2-methylisoborneol) using different organic acids

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**Abstract.** A study on the reduction of off-flavour compounds that cause “earthy” and “musty” organoleptic sensorial characteristic in fresh water systems was conducted. These compounds include geosmin and 2-methylisoborneol (MIB), which are produced as metabolites by some algal and bacterial species. Drinking water and aquaculture commodities affected by these compounds become undesirable to consumers, and market values tend to drop significantly. Triple distilled water samples spiked with approximately  $1 \mu\text{g Kg}^{-1}$  geosmin and  $1 \mu\text{g Kg}^{-1}$  2-methylisoborneol were dehydrated through acidification using two different food grade organic acids (acetic and citric acid), at different concentrations: 0.1, 1.0 and 4.0% (w/w). Levels of geosmin and MIB reduction were determined using Solid Phase Microextraction (SPME) and Gas Chromatographic/Mass Spectroscopic (GCMS) analyses. Results showed that acidification treatments of the off-flavour compounds using varying concentrations of acetic and citric acids significantly reduced the concentrations of geosmin and MIB in the samples. Treatments with 1.0% citric acid and 4.0% acetic acid significantly reduced the concentration of geosmin to concentrations as low as  $0.07 \pm 0.03$  and  $0.09 \pm 0.01 \mu\text{g Kg}^{-1}$  respectively, while the reduction of MIB to concentrations of around  $0.05 \pm 0.01 \mu\text{g Kg}^{-1}$  was achieved at a minimum concentration of 0.1% of either acid used. Previous studies reported that products of the dehydration process of geosmin and MIB produce non-odorous products such as argosmin, and 2-methylenebornane and 1-methylcamphene respectively.

**Key Words:** earthy, musty, acidification, off-flavour reduction.

**Introduction.** Milkfish (*Chanos chanos* Forsskål, 1775) and other aquaculture industries have been experiencing postharvest problems in some freshwater fish culture systems. Cases of off-flavour (undesirable odour and taste) have been reported worldwide causing the water quality and the value of other associated crops to drop significantly. These off-flavour were reportedly caused by the presence of chemical compounds, such as geosmin (trans-1,10-dimethyl-trans-9-decalol) and 2-methylisoborneol (1,2,7,7-tetramethyl-exo-bicyclo-heptan-2-ol or 2-MIB), that are produced as metabolites by some bacterial and algal species living in the culture water (Smith et al 2008; Guttman & van Rijn 2008; Lorio et al 1992; Persson 1980).

These off-flavours, which are perceived to be “earthy” and “musty”, are present in the waters, and accumulate in fish muscles (Gerber 1983; Johnsen & Kuan 1986; Lovell et al 1986; Persson 1980; Maga 1987). Geosmin and 2-MIB have a human detection threshold level in water of up to 0.015 and 0.035  $\mu\text{g Kg}^{-1}$  respectively; however detection threshold levels in fish muscles are also influenced by the lipid content of the fish among other factors (Howgate 2004).

Several postharvest processes have been studied in relation to the removal of geosmin, MIB, and their associated off-flavours (Mills et al 1993; Mireles-DeWitt et al 2007; Yamprayoon & Noomhorm 2000). A previous study by Yamprayoon & Noomhorm (2000) investigated the masking and reduction effect of off-flavours in tilapia (*Oreochromis niloticus*) using different preservations processes such as salting, drying, frying, smoking, microwave heating, marinating and fermentation. Among these processes, acidification either through direct addition of acids or eventual acid formation through fermentation, is one of the more common and traditional methods practiced for fish preservation that yield a significant decrease in the off-flavour compounds in fish.

Although not specifically aimed at reducing off-flavour compounds, acidification of fishery products tainted with geosmin and MIB presents a viable process, since these compounds can be eliminated or reduced given their nature as tertiary alcohols. Through the addition of acids, tertiary alcohols follow the  $S_N1$  reaction wherein the hydroxyl group can easily be removed as  $H_2O$  (dehydration) (McMurry 2010). Assuming that changes in the structure of a particular compound also affect its properties, this simple process can therefore be a potential solution to reduction the off-flavour and odour compounds associated with geosmin and MIB.

In order to better understand the potential of acidification in eliminating off-flavours and odours in fishery products, an initial study to determine the interaction of geosmin and MIB with acids in aqueous solutions is important, especially in relation to the type of acid used and their relative concentrations.

## Material and Method

**Standard solutions.** Analytical grade standard solution of (+/-) Geosmin and 2-Methylisoborneol in methanol (*Supelco® Analytical*) was used as the off-flavour compound; diluted with triple distilled water to come up with a final concentration of approximately  $1 \mu\text{g kg}^{-1}$  and a total mass of 30 g. Sodium chloride (7.5 g) was added for more efficient extraction in Solid Phase Microextraction (SPME). This solution was prepared in 21 replicates, and was divided into 7 groups for the 7 different treatments with 3 replicates.

**Acid treatments.** Pure (99.8%) glacial acetic acid and pure (99.7%) citric acid with three varying concentrations (0.1%, 1.0% and 4.0%) were used as variables in the acidification treatments. Another treatment without acid was used as the negative control. All treatments were done in triplicates. Different concentrations of acid solutions were respectively added to the prepared off-flavour solutions.

**Quantitative analysis.** All samples were mixed thoroughly, and were divided into two parts. Half (~15 mL) of the samples were allocated for the pH analysis, and the other half (~15 mL) was stored in a sealed vial for the Gas Chromatographic analysis.

Effects of the different acid treatments were analyzed using Gas Chromatography/Mass Spectroscopy (GCMS) (*Perkin Elmer Clarus 600*) via SPME method. Such method for off-flavour compound analysis was adapted from the method used by *Supelco® Analytical* for the Solid Phase Microextraction of Odours in Drinking Water, for Analysis by GC/MS (Saito et al 1999; Schrader & Summerfelt 2010).

**Statistical analysis.** One-way analysis of variance was used to evaluate the data derived from the GCMS quantitative analysis, while the Duncan's Multiple Range Test was used to determine the significant differences of the values between treatments, at 5% level of significance.

## Results and Discussion

**Differences in pH values using different organic acid solutions.** Acetic acid at 0.1% concentration gave a pH value of 2.61, while citric acid at the same concentration gave a lower pH value of 1.93 (Figure 1). An inversely proportional trend was observed, wherein as the concentration of the acids increase, the pH values of the acid solutions decreased. The two acids used having the same (%) concentrations, differ in the resulting pH values due to the differences in their dissociation constants.

Acetic acid ( $\text{CH}_3\text{COOH}$ ) has an acid dissociation constant ( $K_a$ ) value of  $1.75 \times 10^{-5}$ ; while citric acid [ $\text{HOOC}(\text{OH})\text{C}(\text{CH}_2\text{COOH})_2$ ] has a  $K_{a1}$  value of  $7.45 \times 10^{-4}$ ,  $K_{a2}$  of  $1.73 \times 10^{-5}$ , and  $K_{a3}$  of  $4.02 \times 10^{-7}$  (Skoog et al 2004). Such different  $K_a$  values resulted in different concentrations of citric acid having significantly lower pH values than that of acetic acid (Figure 1). Citric acid, a triprotic acid can dissociate its first hydrogen atom at  $7.45 \times 10^{-4}$ , while acetic acid, a monoprotic acid, can dissociate its only hydrogen atom at

$1.75 \times 10^{-5}$ . First and second hydrogen atoms of citric acid dissociate faster than the only hydrogen of acetic acid, thus making the former exhibit a lower or more acidic pH.

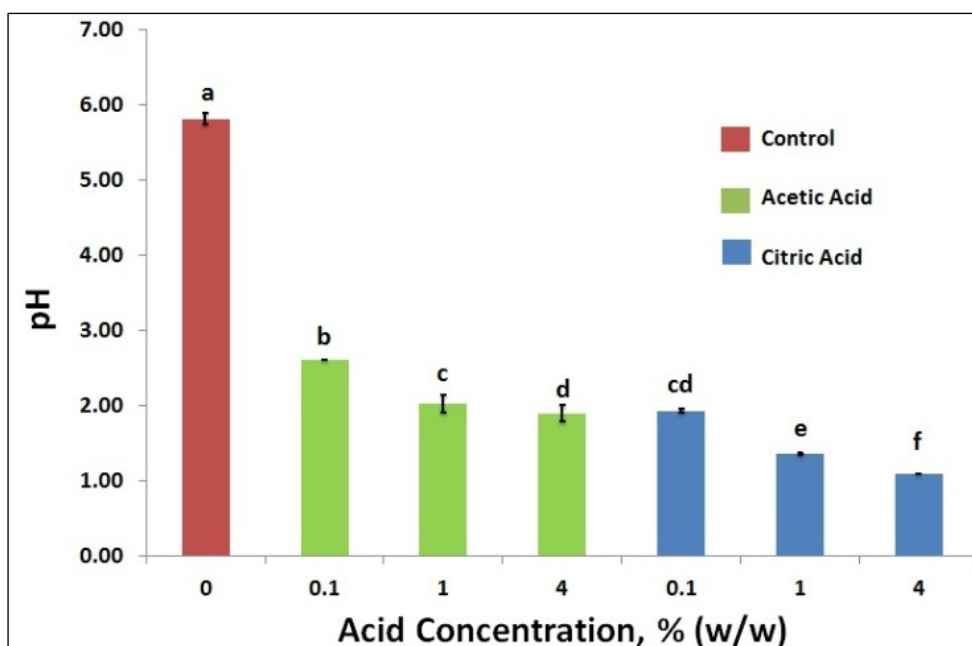


Figure 1. pH values of different acids ( $\pm$  standard deviation) at varying concentrations. Values under different letters are significantly different ( $p \leq 0.05$ ).

**Off-flavour reduction in water.** Using gas chromatographic analyses, the effect of the different acid treatments on the levels of the geosmin and 2-methylisoborneol were evaluated.

**Geosmin.** Concentration of geosmin significantly dropped through the acidification process, as shown in Figure 2. As the concentration of the acids increases, the amount of geosmin was reduced significantly ( $p \leq 0.05$ ). In the acetic acid treated geosmin-spiked water samples, there were no significant differences between the control and at 0.1% acidification treatment, while a significant drop of the geosmin levels were observed at 1.0% and 4.0% acetic acid treatment. In the citric acid treated samples, a significant drop of geosmin levels were observed between the control, the 0.1% and 1.0% acidified treatment, in which values from  $2.14 \pm 0.36$  dropped to  $0.99 \pm 0.32$ , then to  $0.07 \pm 0.03 \mu\text{g Kg}^{-1}$  respectively upon acid treatment. However, no significant difference was observed between the 1.0% and 4.0% citric acid treatment.

Comparing the two acids with similar concentrations, significant differences in the reduction of geosmin were observed except for the 4.0% acid treatment, where there was no significant difference in the amount of geosmin reduced. Thus, reduction of geosmin to its minimum level can be achieved regardless of the acid used (acetic or citric acid), provided acid concentration is greater than or equal to 4.0%. Looking at the pattern and the relationship of pH and the reduction of levels of geosmin, pH lower than approximately 1.90 gave the greatest reduction of geosmin levels in the treatments. As shown in Figure 2, the treatments with 1.0% citric acid, 4.0% acetic acid and 4.0% citric acid had the greatest reduction results which had pH values of  $1.35 \pm 0.02$ ,  $1.89 \pm 0.11$ , and  $1.10 \pm 0.01$  respectively.

Geosmin, trans-1,10-dimethyl-trans-9-decalol, has a bicyclic structure with two methyl groups and an alcohol as substituents. The most probable pathway of geosmin degradation could have been through dehydration. The OH<sup>-</sup> substituent in the molecule binds with the H<sup>+</sup> from the dissociated acid, dehydrating it, and thus resulting to an alteration in the structure of the molecule (Carey & Sundberg 2007). Geosmin molecule without its OH<sup>-</sup> substituent is a different molecule called argosmin (naphthalene,1,2,3,4,4a,5,6,7-tetrahydro-4a,8-dimethyl), which is odourless and flavourless unlike geosmin (Figure 3) (Gerber & Lechevalier 1965; Mills et al 1993;

Schumann & Pendleton 1997). The result of this study shows that such a reaction could have possibly taken place in an acidified geosmin-contaminated water sample. It therefore implies that such dehydration reaction of geosmin to argosmin could potentially be exploited in the attempt to reduce the amount of geosmin in water samples.

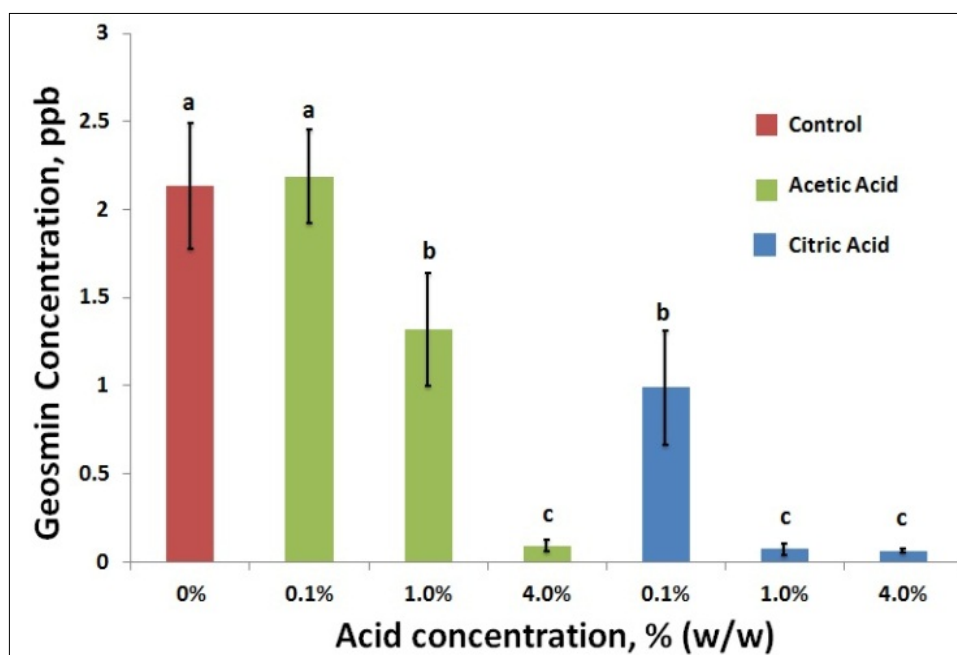


Figure 2. Concentrations of geosmin, expressed as  $\mu\text{g L}^{-1}$  (ppb)  $\pm$  standard deviation, in different acidification treatments. Values under different letters are significantly different ( $p \leq 0.05$ ).

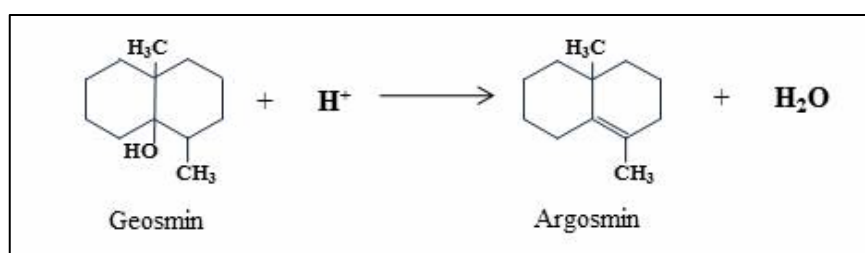


Figure 3. Dehydration process of geosmin.

2-MIB. The concentration of 2-MIB, acidified with acetic and citric acids, significantly dropped (Figure 4). Quantitatively, the levels decreased from  $0.97 \pm 0.18 \mu\text{g Kg}^{-1}$  to  $0.05 \pm 0.01 \mu\text{g Kg}^{-1}$  and  $0.06 \pm 0.04 \mu\text{g Kg}^{-1}$ ; from 0% in the control to 0.1% acetic acid and 0.1% citric acid respectively. Regardless of the acid added (acetic and citric acid) and the concentrations, levels of 2-MIB decreased similarly with no significant difference between treatments (Figure 4). MIB can be effectively reduced at pH levels less than or equal to approximately 2.61, where it is the highest pH recorded among the different acid treatments.

The compound 2-MIB or 1,2,7,7-tetramethyl-exo-bicyclo-heptan-2-ol, upon acidification, may have undergone a dehydration process as well, and produced two probable isomeric compounds, 2-methylenebornane and 1-methylcamphene (1MC) (Figure 5) (Yamprayoon & Noomhorm 2000; Song & O'shea 2007). Such compounds do not reportedly contribute to off-flavour (Mills et al 1993). Either acetic or citric acid, at a minimum concentration of 0.1% could significantly reduce MIB to its minimum detectable level.



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