Predictive modelling and experimental studies on taste-taint as geosmin (GSM) and 2-methylisoborneol (MIB) in farmed barramundi (Lates calcarifer)

by

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DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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EXECUTIVE SUMMARY

Fish farming with Recirculating Aquaculture Systems (RAS) is becoming widespread to fill the demand gap due to diminishing wild caught sea foods. Barramundi fish has a high demand as a premium Australian seafood, and is grown as an RAS farmed-fish. However, the accumulation of 'earthy' or 'muddy' off-flavours due to taint accumulation as geosmin (**GSM**) or 2-methylisoborneol (**MIB**) in the fish-flesh of is a major concern. Inconsistent quality of farmed barramundi has been identified as a major issue in buyer resistance.

Established predictive models for chemical taint in fish-flesh have been based on steady-state assumptions. However, it was thought debatable as to whether a steady-state assumption could be upheld i.e. there was no evidence that the net chemicals exchange is zero across the fish body and RAS water phase.

Against this background, an original, new and quantitative model that predicts the time dependent concentration of taste-taint chemicals as **GSM** and **MIB** in harvested fish-flesh was developed (Hathurusingha & Davey, 2013; Hathurusingha & Davey, 2014; Davey & Hathurusingha, 2014). This model is based on conservation of mass and energy, and thermodynamic processes established in (bio)chemical engineering with chemical uptake and elimination routes into and from the fish considered.

The model was simulated for two RAS species, barramundi (*Lates calcarifer*) and rainbow trout (*Onchorhynchus mykiss*) with independent data ($n \geq 14$) and showed good agreement with experimental observations. A major benefit of this new model is that simulations can be used to investigate a range of growth protocols in RAS farming to minimize taint in fish-flesh. An advantage is that it can readily be simulated in standard spread-sheeting tools by users with a range of sophistication.

Extensive experimental testing of the new model was carried out in both pilot- and commercial-scale plants using low concentrations ($\leq$ 10 mg L^{-1}) of hydrogen peroxide (H_2O_2) as a benign biocide to limit natural occurring taste-taint chemicals in the RAS growth water, and subsequently into the fish-flesh. A dedicated methodology and new dosing apparatus (*ProMinent Fluid Control Pty Ltd*, Germany) for controlled H_2O_2 dosing was developed. The analyses of taste-taint chemicals as **GSM** and **MIB** in water and fish-flesh was carried out with Solid-Phase Micro-Extraction (SPME) followed by Gas Chromatography Mass spectroscopy (GC-MS) (skills training was obtained at both the University of Laval and University of Waterloo, Canada).

Preliminary investigations with a low concentration of H_2O_2 (5 mg L^{-1}) in pilot-scale (2,500 L) studies with barramundi fish demonstrated its potential to mitigate development of

GSM and **MIB** in RAS water. It was found that controlled dosing of low concentrations of H_2O_2 did not impact the pH level in growth waters and was not detrimental to the health and well-being of the fish as fingerlings (0.01 kg) and until harvest at 240 days (0.8 kg). Additional benefits of H_2O_2 as benign biocide include a fish product of whiter colour, an increased dissolved oxygen concentration (C_{OX}) in the growth water, a reduction in the number of gill flukes, and improved particles distribution with increased C:N ratio, and; improved availability of organic carbon in the growth water.

Based on these preliminary investigations H_2O_2 was 'optimised' at a (low) concentration of 2.5 mg L^{-1} as a benign biocide. This was investigated in commercial-scale studies (conducted at *Barra Fresh Farm*, South Australia) for a typical growth of 240 day for barramundi as the selected RAS fish.

The emerging risk methodology of Davey and co-workers (e.g. Chandrakash et al., 2015) was applied for the first time to investigate quantitatively the impact of naturally occurring fluctuations in taste-taint chemicals in the RAS water and their accumulation in the fish-flesh. This predictive approach was justified because of the prohibitively expensive time and analytical costs that experimental studies would have necessitated. A Refined Monte Carlo (with Latin Hypercube) simulation of **GSM** and **MIB** in the growth water (C_W), water temperature (T) and growth time (t) was used to simulate typical RAS farmed barramundi. It was found in RAS farming of barramundi it would be expected some 10.10 % of all 240 day harvests, averaged over the long term, would result in fish with taste-taint as **GSM** above the desired consumer rejection threshold concentration (0.74 µg kg^{-1}) due to natural fluctuations in an uncontrolled RAS environment. For **MIB** this predicted failure rate was 10.56 % (Hathurusingha & Davey, 2016). The vulnerability to taste-taint failure as **GSM** and **MIB** was shown to be principally controlled by the time to fish harvest, and to a lesser extent by concentration and fluctuation of these taint chemicals in the RAS water. This work was of practical benefit because growth time can be readily controlled by farmers. The methodology appears generalizable and therefore is applicable to a range of RAS farmed fish (and possible crustaceans e.g. prawns- *Macrobrachium sp*.).

In extensive commercial-scale RAS studies with barramundi and controlled H_2O_2 dosing, fish grown from fingerlings to harvest at 240 day was investigated. This was to observe an entire production cycle. Results from a H_2O_2 'treated' growth tank (30,000 L) were compared directly with those obtained from an identical 'control' tank (30,000 L). Increased organic matter (three (3) to four (4) times pilot-scale findings) reduced H_2O_2 efficacy through inhibiting generation of reactive oxygen species (ROSs). This is thought to be a consequence of the need to scale (48 times volume) the pilot-scale studies for in-tank mixing.

Analyses of fish-flesh ($n \geq 167$) showed (moderate) predicted exponential correlation between taste-taint concentrations in the fish-flesh and the growth-mass of the fish for both **GSM** and **MIB** as predicted. In addition, the research findings highlighted that accumulation of taste-taint compounds was mainly governed by the combined effect of mass of the fish (m_f) and taste-taint concentrations in the growth water (C_W).

Comparisons between the model predictions and experimental observations showed good agreement over the range of low taste-taint concentration (0 to 2, μg kg^{-1}), especially below the consumer rejection threshold (~ 0.7 μg kg^{-1}). However, a minor anomaly was an over-prediction for greater concentrations (2 to 11, μg kg^{-1}). Current predictions are therefore conservative or 'safe' by about 20 %. Possible reasons for over prediction might be attributed to rapid fluctuation of taste-taint concentration in growth water with growth time and different (exponential) growth constants shown by larger and smaller fish, and; errors in obtaining representative samples from fish-flesh.

Model predictions and experiments further highlighted that the new model could be meaningfully applied to RAS systems with lower variations and/or lower taste-taint concentrations in RAS growth water.

These theoretical and experimental results are the first for RAS farmed fish covering an entire production period to harvest.

Approval for this research was gained from both *The University of Adelaide Animal Ethics Committee Science* and, *Australian Pesticides and Veterinary Medicines Authority* (*see* Appendices F and G).

Research findings will be of immediate benefit to RAS farmers, fish processors and risk analysts in foods processing.

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I hope that the results of my efforts justify the expectations and confidence of the people concerned, and the interest, help, and encouragement of my family, friends and colleagues.

DEDICATION

This thesis is dedicated my mother Mrs Kusumawathie Hathurusingha who passed away one year after starting my PhD research work. She was my inspiration in all my life and unfortunately she is not with us to see the success of my studies. I wish her attainment of supreme '*Nibbana*' according to the Buddhist philosophy.

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