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SHORT COMMUNICATION

Evaluation of geosmin and 2-methylisoborneol off-flavour in smoked rainbow trout fillets using instrumental and sensory analyses

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Taste and odour compounds (TOCs) produced by certain species of cyanobacteria (Smith, Boyer & Zimba 2008) and actinomycete bacteria (Guttman & van Rijn 2008) can accumulate in the flesh of fish and subsequently have significant negative impacts on the aquaculture industry including the following: (1) economic losses to the producers from delayed harvest due to an unpalatable and unmarketable product; and (2) consumer dissatisfaction, which hampers the growth of the industry. Common TOC problems are believed to decrease the sale of channel catfish *Ictalurus punctatus* (Rafinesque) by 30% in the United States of America (Engle, Pounds & van der Ploeg 1995). In Europe, tainting of fish by TOCs has been observed in several countries, e.g. in the French rainbow trout aquaculture industry (Selli, Prost & Serot 2009), and, in the United Kingdom, up to 20% of the trout producers have experienced seasonal problems (Robertson, Hammond, Jauncey, Beveridge & Lawton 2006). Smith *et al.* (2008) cited studies identifying off-flavour occurrence in Nile tilapia, shrimp, Atlantic salmon, rainbow trout, catfish species, American lobster, largemouth bass and white sturgeon. The latter two occurrences involved water recirculation systems.

Water quality and availability impact site location of aquaculture facilities. In many countries, including Denmark, restrictions in water consumption for aquaculture have been implemented. The goal is to reduce the consumption of natural water resources in traditional flow-through aquaculture and increase construction of recirculating production systems. Unfortunately, recirculation of the water increases the risk of accumulation of TOCs, such as geosmin and 2-methylisoborneol (MIB), because these TOCs are slowly degraded by microorganisms in aerobic biofilters (Guttman & van Rijn 2009).

Production of rainbow trout *Oncorhynchus mykiss* (Walbaum) in Denmark requires a 9-month growing season to achieve a fresh-weight of 300–350 g fish. Fat content of the fish is typically about 5%. Most of the rainbow trout produced are salted, smoked over beech chips, vacuum-packed in polyethylene film filled with 30% CO_2 and 70% N_2, and sold in the European market. In Denmark, recent data on geosmin and MIB concentrations in the water of $>1000\ m^3$ concrete production basins confirm 5–10-fold higher TOC levels than in flow-through systems (N. O. G. Jørgensen & B.W. Strobel, unpubl. obs.). Smoked fillets of trout produced in recirculating aquaculture systems (RAS) have occasionally led to consumer complaints about off-flavours, but the levels and composition of TOCs in the flesh of the fish cultured in these systems have not been determined. In addition, there has not been an evaluation of salting and smoking the fillets on flavour quality due to 'masking' of the off-flavours. The purpose of this current study was to quantitatively assess if smoked trout fillets from the recirculated systems retained volatile aromatics contributing to the off-flavours and, if so, the potential benefits of smoking and salting on improving flavour quality by masking any residual TOCs.

For this study, rainbow trout were bred in a 1200 m^3 RAS supplied with groundwater, and biofilter techniques were used for water treatment (cleaning), with approximately 50% of the water volume replenished daily. The commercial system and exact location in Denmark is not identified by request of the owner. Standard Danish commercial methods for feeding, grow-out, harvesting and processing were used to produce the final product of smoked trout fillets. The smoked fillets were kept at $-20\,^{\circ}C$ for about 2 months before analyses of off-flavours and the common TOC compounds geosmin and MIB.

Before conducting instrumental analysis, frozen fillets (sampling set of 12) were partially thawed. For each fillet, one 20 g portion was cut from the anterior end of the fillet and used to obtain distillate with microwave distillation using the procedures of Lloyd and Grimm (1999). Distillates were analysed using headspace SPME/GC/MS as previously described by Lloyd, Lea, Zimba and Grimm (1998) and as modified by Schrader, Nanayakkara, Tucker, Rimando, Ganzera and Schaneberg (2003).

For sensory analysis, the sensory panel consisted of six assessors that had been selected, tested and specifically trained in descriptive analysis (ISO 11035) of rainbow trout (Hyldig 2009a). The vocabulary for the sensory profiling was developed during four sessions using samples of smoked rainbow trout with known content of MIB and geosmin, i.e. the compounds were not added. The first session was qualitative, i.e., to develop a list of attributes describing odour, flavour and texture of rainbow trout. The following three sessions were quantitative, i.e. the assessors were trained to evaluate the descriptors on a linear scale. Each attribute was evaluated using a 15-cm unstructured linear scale with two anchor points that were 'little' and 'much' of attribute intensity. The anchor points were placed 1.5 and 13.5 cm from 0 on the scale (Meilgaard, Civille & Carr 2007). The sensory attributes analysed were the following: (1) odour (e.g. smoke, wood tar, sweet, sourish, fusty/mouldy and muddy); and (2) flavour (e.g. smoke, sweet, salty, sourish, oily, earthy, mouldy/fusty and muddy); (3) texture (juicy and fibrousness); and (4) light/dark colour for appearance (Hyldig 2009b). The evaluations were performed in separated booths under normal daylight and at ambient temperature. The assessors used water and flat bread to clean the palate between samples. Each sample was minced and mixed before subsampling into individual porcelain bowls covered with porcelain lids. The fillet size was insufficient for replicate analyses by each assessor. A sample without any sensory detectable MIB and geosmin was used as reference.

The instrumental analyses demonstrated that all 12 smoked trout samples contained both MIB and geosmin. The levels of MIB were at least one order of magnitude greater than geosmin levels in each respective fillet; MIB was the dominant odour component (Fig. 1). The MIB concentrations ranged from 4.8 to 19.7 $\mu g\,kg^{-1}$ while geosmin ranged from 0.27 to 0.59 $\mu g\,kg^{-1}$.

Observations by the sensory panel confirmed the presence of TOC in the smoked fillets and mainly attributed to the off-odour and off-flavour from both MIB (mouldy/fusty) and geosmin (muddy) (Fig. 2). The intensity of the off-flavour from MIB and geosmin was significantly higher than the other odours in most samples, but especially noteworthy was the intense fusty/mouldy odour originating from MIB in sample 'Fish 8'. Interestingly, Fish 4 and 8 both had a high content of MIB (statistically identical) (Fig. 1), yet Fish 8 had a higher score for mouldy/fusty flavour and odour (Fig. 2). Possibly, the saltier flavour and smoked (wood tar) odour of Fish 8 masked the mouldy/fusty attributes.

Sensory profiles illustrating the tested odour, flavour and texture attributes are shown for a low, medium and high score fish with respect to flavour in Fig. 3. The sensory plots indicate that even though the intensity of smoke and wood tar was relatively high, the odours and flavours from MIB and geosmin were easily detectable and identifiable. In previous studies with channel catfish, MIB levels were found to in some cases to vary greatly between the two fillets from the same fish (K. K. Schrader, unpubl. obs.). In the current study, different fillets from the same fish were used for sensory analysis and instrumental analysis, and this reason may account for some of the differences (e.g. Fish 4) between the intensity of MIB-related off-flavour (Fig. 2) compared with the MIB levels in the same fish (Fig. 1).

The levels of MIB in the sampled trout fillets were typically 20-fold above geosmin levels. This difference was also reflected in the sensory profiles that were dominated by the mouldy/fusty flavour and

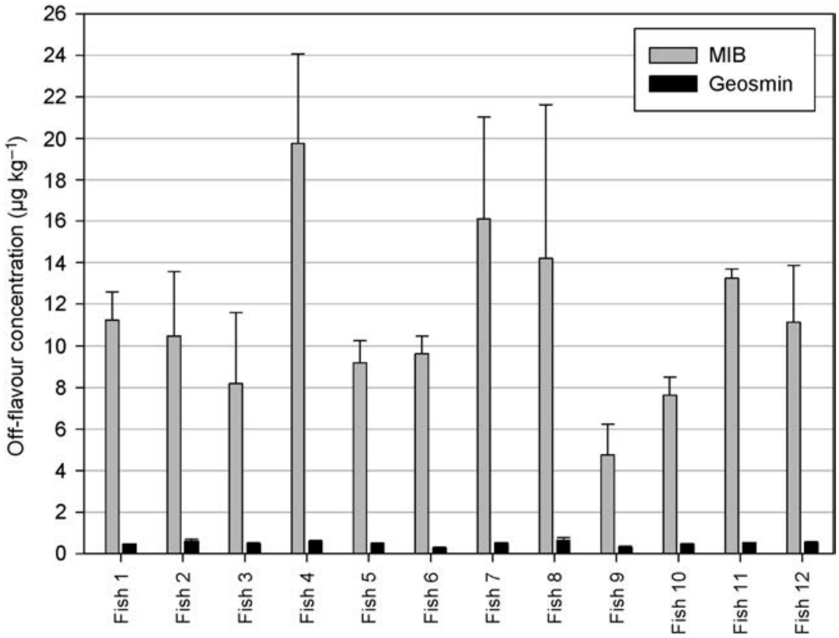

Figure 1 Geosmin and 2-methylisoborneol (MIB) levels in rainbow trout fish fillets from a commercial Danish production facility. Mean concentrations ± 1 SD shown (n = 3).

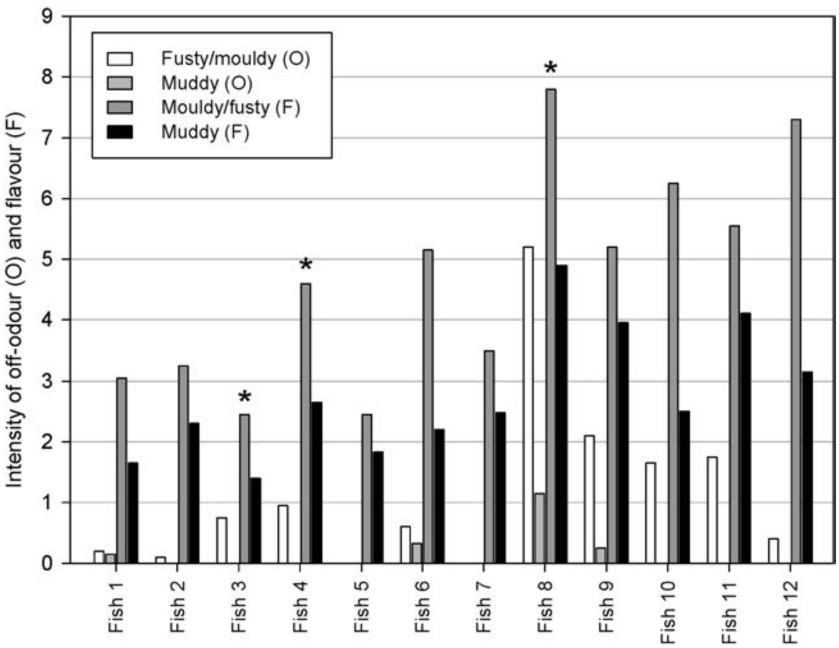

Figure 2 Intensity scores from human sensory evaluation of off-odour (O) and off-flavour (F) from 2-methylisoborneol (MIB) (fusty/mouldy) and from MIB and geosmin (muddy). Asterisks (*) indicate fish for which sensory profiles are shown in Fig. 3.

odour that are characteristic of MIB. Geosmin was previously reported to be the major TOC in rainbow trout (Robertson *et al.* 2006), and our study is the first to report on the high levels of MIB in cultured rainbow trout. Unfortunately, the levels of geosmin and MIB could not be measured analytically in the basin

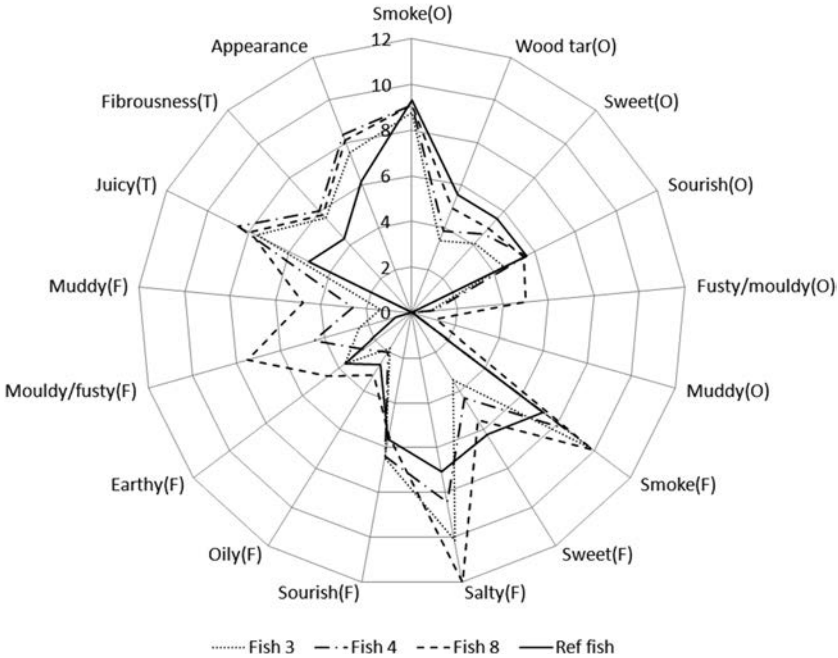

Figure 3 Sensory profiles for Fish 3, 7, 8 and a reference fish. Intensity of the sensory attributes ranges from 0 (no score; centre of plot) to 12 (maximum score; perimeter of plot). The letters 'O', 'F' and 'T' refer to odour, flavour and texture respectively.

water, but the higher MIB levels in the fish flesh indicates that MIB was likely at a substantially higher concentration than geosmin.

Geosmin and MIB are produced by certain microorganisms that are abundant in aquaculture systems, such as cyanobacteria and actinomycetes (Zaitlin & Watson 2006; Smith *et al*. 2008; Schrader & Summerfelt 2010). Recently, Guttman and van Rijn (2008) observed a large release of MIB from aerobic biofilter material in tilapia ponds (up to 175 ng L^{-1}) and concluded that the compound was produced by *Streptomyces* bacteria. In Danish rainbow trout basins with water recirculation, levels of geosmin and MIB in water of 30–40 and 5–15 ng L^{-1}, respectively, have typically been observed (N. O. G. Jørgensen & B. W. Strobel, unpubl. obs.).

Variable thresholds for human sensory detection of MIB and geosmin are reported for fish. For channel catfish, Grimm, Lloyd and Zimba (2004) reported a threshold of 0.1–0.2 μg kg^{-1} for MIB and 0.25–0.5 μg kg^{-1} for geosmin while Robertson, Jauncey, Beveridge and Lawton (2005) suggested a threshold of 0.7 μg kg^{-1} for MIB and 0.9 μg kg^{-1} for geosmin in rainbow trout. Thus, depending upon the applied threshold level from these two studies, the level of MIB in our sampled trout was 10–25 times or 80–200 times above the detection threshold while geosmin levels in the fish appeared to be just below the threshold.

It is well established that off-flavours are assimilated into fish tissue, with removal being a slow process [reviewed in Smith *et al*. (2008) and Tucker (2000)]. Natural depuration efforts can be slow and influenced by temperature, fat content of fish, as well as the amount of off-flavour compound accumulated (Dionigi, Johnsen & Vinyard 2000). Therefore, postharvest approaches in mitigating geosmin and MIB-related off-flavour can be useful when the taint is still present. Bett, Ingram, Grimm, Vinyard, Boyette and Dionigi (2000) demonstrated that masking of MIB and geosmin off-flavour in catfish with spices such as lemon-pepper and Cajun mixes is effective. In this study, we anticipated that processed smoking of trout fillets would result in lowered sensory perception of off-flavour by potential removal of volatile aromatics during the smoking process and the accumulation of additional intense odorous compounds from the beech smoke into the fillets. Beech wood smoke largely consists of lignin degradation products, primarily hemicelluloses and cellulose, with minor contribution by furans from lignin breakdown but no terpenes (Omrani, Masson, Pizzi & Mansouri 2008). As demonstrated in our study, the high detected intensities of odours and flavours such as smoke and wood tar from the smoking process did not hamper sensory detection of the strong fusty/mouldy and muddy odours and flavours. Therefore, the smoking process was not effective in reducing or masking the respective odours and flavours of geosmin and MIB at the levels detected in the trout flesh.

As more recirculation production systems are established, management to prevent off-flavour occurrence is essential to maintain customer acceptance of product quality. Development of alternative processing lines (e.g. spice addition) may provide alternatives in situations when off-flavour fish are inadvertently found.

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