Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 107 (2008) 1605–1615
Food Chemistry
www.elsevier.com/locate/foodchem

Biometric, nutritional and sensory changes in intensively farmed Murray cod (*Maccullochella peelii peelii*, Mitchell) following different purging times

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Received 8 May 2007; received in revised form 27 July 2007; accepted 12 September 2007

Abstract

A period of purging before harvesting is common practice in intensive aquaculture to eliminate any possible off flavours from the fish. The present study was conducted to evaluate the biometrical, nutritional and sensory changes in intensively farmed Murray cod (*Maccullochella peelii peelii*) after 0, 2 and 4 weeks of purging. After the main biometric parameters were recorded, fish were analysed for proximate, fatty acid composition and flavour volatile compounds. A consumer preference test (triangle test) was also conducted to identify sensorial differences that may affect the consumer acceptability of the product.

Fish purged for 2 and 4 weeks had a significant weight loss of 4.1% and 9.1%, respectively, compared to unpurged fish, whilst perivisceral fat content did not change. The concentration of saturated (SFA), monounsaturated (MUFA) and highly unsaturated (HUFA) fatty acids were not significantly affected by purging time, while polyunsaturated fatty acids (PUFA), $n-3$ and $n-3$ HUFA were significantly higher ($P < 0.05$) in purged fish compared to unpurged fish. Consumers were able to detect differences between the purged and unpurged fish ($P < 0.05$) preferring the taste of the purged fish. However, consumers were unable to distinguish between fish purged for 2 and 4 weeks.

This study showed that a 2 weeks purging period was necessary and sufficient to ameliorate the final organoleptic quality of farmed Murray cod. With such a strategy the nutritional qualities of edible flesh are improved while the unavoidable body weight loss is limited.

Keywords: Flavour volatile compounds; Sensory evaluation; Purging; Starvation; Fatty acids; Product quality

1. Introduction

Murray cod, *Maccullochella peelii peelii* (Mitchell), is the largest Australian native, carnivorous, warm water fish. It resembles the Chinese Mandarin fish, *Siniperca chuatsi* (Basilewski), which is considered one of the most valued freshwater species commercially cultured (De Silva, Gunasekera, & Ingram, 2004), and on the Australian market is highly valued by some ethnic groups. In recent years there has been much interest in Murray cod culture due to its large size, fast growth rate, flesh quality and suitability to intensive culture. Currently Murray cod supports a well established and growing aquaculture industry within Australia (Ingram, De Silva, & Gooley, 2005), which is supported also by the closure of the wild fishery in 2002. Nationally, it is also very important for conservation purposes. Intensive aquaculture practices depend on an

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doi:10.1016/j.foodchem.2007.09.079

externally supplied wholesome diet which has the potential to impact the nutritional and sensorial quality of farmed fish (De Silva & Anderson, 1995). Therefore, the large-scale retail and the fish processing industry are imposing quality criteria for the end product (Greenhalgh, 2001; Rasmussen, 2001; Rønsholdt & McLean, 1999). The strategies used so far to reduce fat content, manipulate the fatty acid make up and improve the final eating quality of fish fillet are: a period of starvation (Einen & Thomassen, 1998; Regost, Arzel, Cardinal, Laroche, & Kaushik, 2001), a period of feed restriction (Pirhonen & Forsman, 1998), the use of a specific finishing diet (Rasmussen, Ostenfeld, Rønsholdt, & McLean, 2000), or the transfer to an extensive culture system in the final part of the production cycle (Turchini, Mentasti, Crocco, et al., 2004).

Intensively farmed Murray cod have been reported to contain a relatively high lipid content and optimal fatty acid composition from a human nutritional point of view (Palmeri, Turchini, & De Silva, 2007). Although Murray cod is extremely well regarded as a table fish, one of the problems that compromise the marketability of intensively farmed specimens to a wider spectrum of consumers is the occasional presence of off flavours in its flesh.

The presence of off flavours in fish is a recurring problem in aquaculture (Robin, Cravedi, Hillenweck, Deshayes, & Vallod, 2006; Schrader, de Regt, Tidwell, Tucker, & Duke, 1998) and it can be very detrimental to marketing of the product (Dionigi, Johnsen, & Vinyard, 2000). Recirculating aquaculture systems (RAS) have been developed to maximise fish output and to minimise water discharge. To date, state of the art systems can operate with only 5–10% daily water discharge without affecting growth (Masser, Rakocy, & Losordo, 1999; Pir.Sa, 2003). As a consequence, accumulation of metabolic by products, such as faeces, and uneaten feed within the system could lead to an accumulation of unpleasant flavours. However, such off flavours are not harmful to people and can be eliminated, though at a slower rate than they have been accumulated (Tucker, 2000) through purging in running water (Dionigi et al., 2000). The purging period can vary from a few days to many weeks depending, amongst other factors, on the intensity and origin of the off flavour (Dionigi et al., 2000).

To date, there are not set finishing/purging protocols for Murray cod producers in Australia. The aims of the present study were to evaluate the effects of different purging time periods on selected biometrical, flesh quality and flavour characteristics of intensively farmed Murray cod and to evaluate consumer's preferences in relation to the purging period.

2. Materials and methods

2.1. Experimental fish and purging facility

Average market size fish (~700 g) were sourced from the stock of the intensive RAS located at Deakin University, Warrnambool, Australia. During the final stages of the grow-out period the fish were fed a commercially extruded diet (Nova, Skretting, Tasmania, Australia: moisture = 70 mg g^{-1}, crude protein = 450 mg g^{-1}, total lipid = 220 mg g^{-1}, ash = 80 mg g^{-1}, energy = 22.4 kJ/g).

Individual weight and length to the nearest g and cm, respectively, of harvested fish were determined, and allocated (20 fish per tank, 3 tanks per treatment) to six purging tanks at 2 weeks intervals to allow final simultaneous harvest, that is, fish to be purged for 4 weeks were stocked first, followed after 2 weeks by fish to be purged for 2 weeks. Control fish, those that did not undergo any purging, were harvested directly from the grow-out tanks the day of the final sampling. The purging system comprised 6 circular 600 l tanks part of a 15 tanks recirculating system with a total volume of approximately 15,000 l. The water (de-chlorinated town water) was exchanged at a rate of 2000 l day^{-1}. Salinity was kept between 5 and 10 g l^{-1} and temperature at ~17 °C. All the other water quality parameters were adequate to the culture of this species (Ingram, 2000).

2.2. Sampling procedure

All procedures implemented in the present study were approved by the Deakin University Animal Ethic Committee. Five fish per replicate were harvested, culled by cutting the main arterial vessel in the throat and put in a drum containing ice slurry until no movement was observed. They were then removed from the ice, dried with a paper towel, filleted and individually vacuum packed using an Orved Best Vacuum (Musile di Piave, Italy) machine and kept frozen at −20° C until needed. Prior to analysis, fillets were pooled according to replicate allocation and homogenised using a mini food processor (Black & Decker, Model BMC100, China).

2.3. Biometric parameters

The main biometric parameters included total length (TL), total weight (TW), somatic weight (SW), liver weight (LW), fillet weight (FW), viscera weight (VW) and perivisceral fat weight (PFW). All weights were in g and length in cm.

The following parameters were also calculated:

Fulton's condition factor: $K = (TW \div L^3) \times 100$.
Hepatosomatic index: HSI (%) = (LW ÷ TW) × 100.
Visceral fat index: VFI (%) = (PFW ÷ TW) × 100.
Dress-out percentage (%) = (SW ÷ TW) × 100.
Fillet yield (%) = (FW ÷ TW) × 100.

2.4. Proximate composition of diets and muscle

Proximate composition of diets and muscle was determined as previously described (Palmeri et al., 2007) using standard methods (AOAC, 2006; codes 930.15; 942.05; 955.04). Briefly, moisture was determined by drying sam-

ples in an oven at 80 °C to constant weight; protein content using Kjeldahl nitrogen × 6.25 using an automated Kjeltech 2300 (Foss Tecator, Höganäs, Sweden), and lipid by chloroform:methanol (2:1) extraction according to Folch, Lees, and Sloane-Stanley (1957) as modified by Ways and Hanahan (1964). Ash content was determined by incinerating samples in a muffle furnace (Wit, C&L Tetlow, Australia) at 550 °C for 18 h. All analyses were performed in duplicate.

2.5. Fatty acid analysis

The quantification of fatty acids was conducted as previously reported in our laboratory (Palmeri et al., 2007; Turchini, Francis, & De Silva, 2006a). Briefly, after extraction, fatty acids were esterified into methyl esters using the acid catalysed methylation method (Christie, 2003), and followed by the methods previously used in the laboratory. The internal standard used was 23:0 (Sigma–Aldrich Inc., St. Louis, MO, USA) and fatty acid methyl esters were isolated and identified using a Shimadzu GC 17A (Shimadzu, Chiyoda-ku, Tokyo, Japan) equipped with an Omegawax 250 capillary column (30 m × 0.25 mm internal diameter, 25 μm film thickness, Supelco, Bellefonte, PA, USA), a flame ionisation detector (FID), a Shimadzu AOC-20i auto injector, and a split injection system (split ratio 50:1). The temperature program was 150–180 °C at 3 °C min^{-1}, then from 180 to 250 °C at 2.5 °C min^{-1} and held at 250 °C for 10 min. The carrier gas was helium at 1.0 ml min^{-1}, at a constant flow. Each of the fatty acids was identified relative to known external standards. The resulting peaks were then corrected by the theoretical relative FID response factors (Ackman, 2002) and quantified relative to the internal standard.

2.6. Computation for fatty acid mass balance

A computation for the fatty acids mass balance was done by calculating the estimated difference (in mg) of individual fatty acids in the fillet at the beginning and the end of the purging period. It was therefore possible to estimate, by difference, the individual fatty acids loss throughout the different purging time. The initial fillet recovery rate and initial lipid content values used in the estimation were obtained from data relative to unpurged fish.

2.7. Flavour volatile compounds analysis

The volatile compounds analysis was performed as previously described (Turchini, Giani, Caprino, Moretti, & Valfrè, 2004; Turchini, Mentasti, Caprino, et al., 2004; Turchini, Moretti, Mentasti, Orban, & Valfrè, 2007) on five fillets from each experimental tank; each analysis was carried out in duplicate. After the fillets were minced, 10 g of sample were placed in a 250 ml flask with 100 ml of purified water (Millipore, Bedford, MA, USA) and a 10 μl solution of undecane (2 mg ml^{-1}) was added as internal standard and subjected to Simultaneous Distillation–Extraction (micro SDE apparatus, Chrompack, Middelburgh, NY, USA) for 2 h. All reagents and solvents were from Merck (Darmstadt, Germany). Compounds were analysed in an Agilent 6890 Series GC system coupled to a 5973N mass selective detector. The separation was performed on a MDN-5 capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness, Supelco, Bellefonte, PA, USA). Carrier gas was helium with a linear flow rate of 1 ml min^{-1}. The oven temperature program was: 35 °C held for 1 min, from 35 to 60 °C at 120 °C min^{-1}, then from 60 to 280 °C at 3 °C min^{-1}. Samples of 1 μl were injected in splitless mode (purge flow 19 ml min^{-1} at 1 min). Mass spectra were obtained under electronic ionisation (EI) condition at 70 eV in the 35–300 amu range. Ion source was held at 230 °C and quadrupole at 150 °C. Identification of compounds was based on mass spectra from library database (NIST 98, WILEY 275) and comparing GC retention times and Kovàtz retention index (RI) with those of known standards. The RI was calculated for comparison of retention data from the literature (Castello, 1999). Data were recorded and analysed with the HP Chemstation Software. The data are expressed as percentage of the total volatile compounds.

2.8. Sensory evaluation

A consumer preference test was conducted over three consecutive days with a total of 34 subjects recruited from the local community (15 males, 19 females: average age 37 ± 2.1). All subjects agreed to participate and provided informed consent on an approved Institutional Review Board form. The participants, all regular fish eaters, were asked to refrain from eating, smoking, drinking or chewing gum for 1 h prior to testing. The fish were defrosted for 2 h at room temperature, filleted, and the upper portion of the fillet cut into four individual portions. These portions (weight 13.92 ± 0.44 g) were then cooked in individually wrapped aluminium foil containers in two preheated fan forced oven at 200 °C for 10 min (both ovens had been previously calibrated to allow for temperature discrepancies). No flavouring or spices were added to the fish.

A triangle forced choice procedure (Meilgaard, Civille, & Carr, 1999) was used to determine if subjects could distinguish between samples of Murray cod purged for 0, 2 or 4 weeks. In any one session only two treatments were compared (e.g. 2 versus 4 weeks purging) resulting in three separate sessions to cover all possible combinations. During a session, each subject was presented with a set of three samples, two identical ones and a different one (triangle test). Each sample was identified by a 3-digit code and the order of presentation was randomised and could have been any of six possible combinations (XXY, XYY, XYX, YXX, YYX or YXY). Participants were requested to determine which sample was the odd one and also to determine which sample/s was/were more acceptable. They were finally asked to give their opinion on the overall quality of the fish in terms of texture, appearance and moisture.

2.9. Statistical analysis

Principal component analysis (PCA) was used to observe the parameters retaining the maximum amount of variability present in the data set and to observe the similarity between groups. This method provides a new set of variables obtained (principal components: PCs) as a linear combination of the original descriptors. These PCs allow an easy representation for visual inspection of the data in a two-dimensional diagram to point out patterns hidden in the dataset. The Unscrambler 9.6 software for Windows was used to perform PCA.

Data are reported as mean ± SEM. After normality and homogeneity of variance were confirmed, one way analysis of variance (ANOVA) was used to determine differences between means. Differences were considered statistically significant at $P < 0.05$. Data were subject to Duncan's post hoc test where differences were detected for homogenous subsets. All statistical analyses were performed using SPSS (SPSS Inc. Chicago, Illinois) v.11.5 for Windows. The results of the consumer evaluation were analysed using Triangle Test for Difference tables in Meilgaard et al. (1999).

3. Results

3.1. Diet composition

All fish were fed the same commercial diet in the months prior to the study. As such, no differences, chemical or organoleptic, are expected to be feed related. The proximate composition and fatty acid profile of the commercial diet are shown in Table 1.

3.2. Biometric parameters

The biometric data relating to Murray cod before and after purging are shown in Table 2. Fish that underwent purging for 2 (2W) and 4 weeks (4W) had a weight loss of 4.1 ± 0.7% and 9.1 ± 0.6%, respectively.

The hepatosomatic index (HSI) was significantly lower ($P < 0.05$) in purged fish (0.9 ± 0.06% in 2W and 0.7 ± 0.04% in 4W) compared to unpurged fish (2.0 ± 0.13%). The dress-out percentage of 2W and 4W increased significantly ($P < 0.05$), compared to 0W, from 89.5 ± 0.36% in 0W to 91.9 ± 0.56% in 2W and 92.5 ± 0.29% in 4W. The fillet yield, on the other hand, decreased significantly ($P < 0.05$) from 41.9 ± 0.53% in 0W to 36.3 ± 0.62% and 35.1 ± 0.88% in 2W and 4W, respectively.

Condition factor (K) and visceral fat index (VFI) remained fairly constant throughout the duration of the trial.

3.3. Proximate and fatty acid composition of the fillets

The 2 and 4 weeks purging did not cause a noteworthy effect on the proximate composition of the fillets (Table 3). Moisture, protein, lipid and energy content did not show

Table 1
Proximate composition (mg g^{-1}), energy and fatty acid profile (% of total fatty acids) of the commercial diet as fed (w/w)

Proximate[a]	Diet[c]
Moisture	65.8
Protein	446.9
Lipid	218.7
Ash	79.9
NFE[d]	18.9
Energy[e]	22.4
Fatty acids[b]	
14:0	4.8
16:0	22.0
18:0	5.7
16:1$n-7$	6.8
18:1$n-9$	24.9
18:1$n-7$	2.8
20:1[f]	1.1
18:2$n-6$	8.0
20:4$n-6$	0.7
18:3$n-3$	1.1
18:4$n-3$	1.4
20:5$n-3$	8.5
22:5$n-3$	1.0
22:6$n-3$	6.0
∑SFA	33.8
∑MUFA	36.2
∑PUFA	29.5
∑HUFA	16.9
∑$n-3$ PUFA	18.4
∑$n-6$ PUFA	9.3
∑$n-3$ HUFA	15.9
∑$n-6$ HUFA	1.0
$n-3/n-6$	2.0

[a] Moisture, protein, lipid and ash expressed as mg g^{-1}. Energy expressed as kJ/g^{-1}.
[b] Only principal fatty acids have been reported.
[c] Skretting Nova ME 11 mm, Tasmania, Australia.
[d] NFE = Nitrogen Free Extract – calculated by difference.
[e] Calculated on the basis of 23.6, 39.5 and 17.2 kJ/g^{-1} of protein, fat and carbohydrate, respectively.
[f] 20:1 represents the sum of 20:1 isomers (20:1$n-9$ and 20:1$n-11$).

Table 2
Biometric and yields data of Murray cod purged for 0, 2, and 4 weeks

	Treatment		
	0 weeks	2 weeks	4 weeks
Initial mean weight[A]	746.3 ± 27.0	655.6 ± 16.6	655.9 ± 21.5
Final mean weight[A]	746.3 ± 27.0[b]	628.6 ± 15.7[ab]	595.9 ± 19.0[a]
Weight loss[A]	–	27.0 ± 5.34[a]	59.9 ± 3.28[b]
Weight loss[B]	–	4.1 ± 0.7[a]	9.1 ± 0.6[b]
HSI[D]	2.0 ± 0.13[b]	0.9 ± 0.06[a]	0.7 ± 0.04[a]
K[C]	1.5 ± 0.05	1.4 ± 0.03	1.4 ± 0.05
VFI[E]	4.6 ± 0.44	5.1 ± 0.36	4.2 ± 0.32
Dress-out[B]	89.5 ± 0.36[a]	91.9 ± 0.56[b]	92.5 ± 0.29[c]
Fillet yield[B]	41.9 ± 0.53[b]	36.3 ± 0.62[a]	35.1 ± 0.88

Values with the same superscript in each row are not significantly different ($P > 0.05$).
[A] Value in g.
[B] Value in %.
[C] Condition factor.
[D] Hepatosomatic index.
[E] Visceral fat index.

Table 3
Proximate composition (mg g^{-1}) (w/w basis) and energy content (kJ/g^{-1}) of the fillets of Murray cod purged for 0, 2 and 4 weeks

	Treatment		
	0 weeks	2 weeks	4 weeks
Moisture[A]	762.5 ± 0.83	771.4 ± 0.43	776.3 ± 0.16
Protein[A]	192.7 ± 0.40	186.7 ± 0.18	178.3 ± 0.56
Lipid[A]	39.8 ± 0.44	38.2 ± 0.28	43.6 ± 0.42
Ash[A]	2.7 ± 0.00[b]	2.4 ± 0.01[a]	2.4 ± 0.01[a]
Energy[B]	6.12	5.92	5.93

Values are mean ± SEM.
Values with the same superscript in each row are not significantly different ($P > 0.05$).
Values are average of 5 fish per replicate.
[A] Moisture, protein, lipid and ash expressed as mg g^{-1}. Energy expressed as kJ/g^{-1}.
[B] Calculated on the basis of 23.6, 39.5 and 17.2 kJ/g^{-1} of protein, fat and carbohydrate, respectively.

Table 4
Fatty acid composition (mg g^{-1} lipid) of fillets of Murray cod purged for 0, 2 and 4 weeks

	Treatment (mg g^{-1})		
	0 weeks	2 weeks	4 weeks
14:0	36.7 ± 1.14	39.6 ± 1.95	39.4 ± 0.30
16:0	155.0 ± 3.35	165.6 ± 5.78	166.5 ± 4.84
16:1$n-7$	42.1 ± 1.47[a]	57.0 ± 2.75[b]	53.8 ± 5.12[ab]
18:0	37.6 ± 0.52	35.2 ± 3.14	39.4 ± 0.59
18:1$n-9$	176.0 ± 3.98	193.6 ± 10.59	191.4 ± 7.95
18:1$n-7$	26.9 ± 0.97	25.7 ± 2.31	29.7 ± 1.08
18:2$n-6$	63.0 ± 1.40	68.9 ± 3.78	66.6 ± 2.99
18:3$n-3$	8.2 ± 0.31	9.9 ± 0.41	9.0 ± 0.23
18:4$n-3$	9.4 ± 0.63	11.1 ± 1.10	11.6 ± 0.41
20:1[A]	7.2 ± 0.03	8.2 ± 0.41	8.0 ± 0.80
20:4$n-6$	9.8 ± 0.89	10.1 ± 1.25	9.5 ± 1.16
20:5$n-3$	55.3 ± 1.07[a]	60.8 ± 1.70[b]	59.8 ± 0.80[b]
22:5$n-3$	30.5 ± 0.70[a]	32.8 ± 0.61[b]	31.6 ± 0.22[ab]
22:6$n-3$	72.8 ± 1.37	74.9 ± 3.48	77.8 ± 0.57
SFA	237.6 ± 5.20	247.3 ± 8.86	252.8 ± 4.87
MUFA	262.2 ± 5.95	297.9 ± 16.56	292.7 ± 14.41
PUFA	276.6 ± 2.13[a]	295.7 ± 7.96[b]	292.2 ± 3.56[ab]
HUFA	186.8 ± 1.03	196.3 ± 5.51	195.0 ± 1.50
$n-3$	188.0 ± 1.10[a]	200.0 ± 5.50[b]	201.3 ± 0.85[b]
$n-6$	85.5 ± 1.06	92.8 ± 2.54	87.9 ± 2.69
$n-3$ HUFA	170.5 ± 0.27[a]	179.9 ± 4.15[b]	180.7 ± 0.45[b]
$n-6$ HUFA	16.3 ± 0.76	16.4 ± 1.36	14.5 ± 1.05
$n-3/n-6$	2.2 ± 0.02[ab]	2.2 ± 0.01[a]	2.3 ± 0.06[b]
Total FA[B]	776.4 ± 15.24[a]	840.9 ± 27.56[b]	837.7 ± 23.84[b]

Only principal fatty acids have been reported.
Values in the same row with the same superscripts are not significantly different ($P > 0.05$).
[A] 20:1 represents the sum of 20:1 isomers (20:1$n-9$ and 20:1$n-11$).
[B] Total FA (%) per g of lipid.

any significant difference amongst treatments ($P > 0.05$), while ash was higher in unpurged fish ($P < 0.05$).

There were no major differences amongst individual saturated and monounsaturated fatty acids profiles of unpurged and purged fish, with palmitoleic acid (16:1$n-7$) being the only monounsaturated to be significantly higher in 2W fish ($P < 0.05$). Eicosapentaenoic acid (EPA, 20:5$n-3$) was significantly higher ($P < 0.05$) in 2W and 4W, while there were no differences ($P > 0.05$) in arachidonic acid (ArA, 20:4$n-6$) and in docosahexaenoic acid (DHA, 22:6$n-3$). Fish purged for 2 weeks also had the highest ($P < 0.05$) PUFA concentration amongst all treatments (295.7 ± 7.96 mg g^{-1} lipid compared to 276.6 ± 2.13 mg g^{-1} in W0 and 292.2 ± 3.56 mg g^{-1} in W4). Total saturated (SFA), monounsaturated (MUFA) and highly unsaturated fatty acids (HUFA; fatty acids with 3 ethylenic bonds with 20 or more carbon chains, namely 20:4$n-6$, 20:5$n-3$, 22:5$n-3$, 22:6$n-3$) did not show significant differences ($P > 0.05$) between treatments. However, $n-3$ HUFAs were highest in 2W and 4W ($P < 0.05$). The data relative to the fatty acid are given in Table 4.

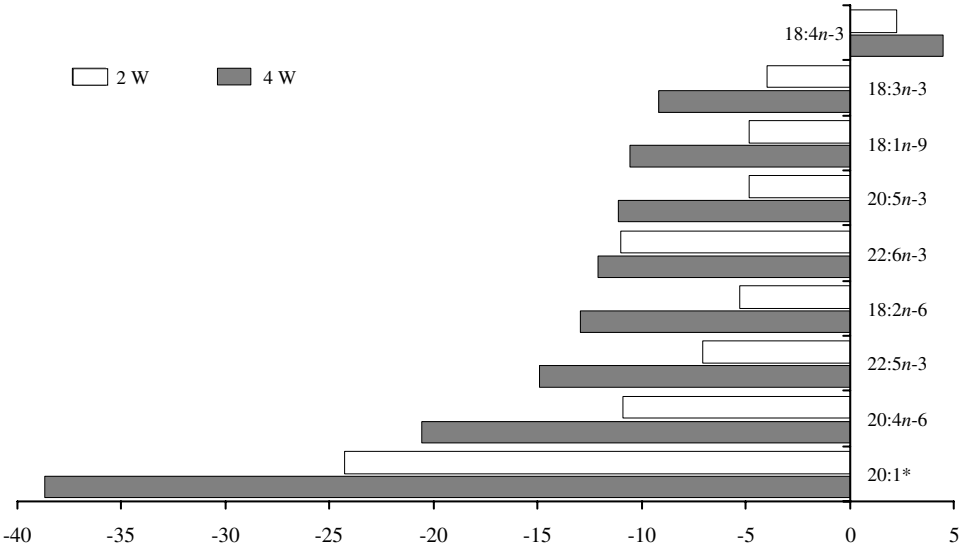

Fig. 1. Estimated total fatty acid loss (mg), in the whole fillet of fish purged for 2 and 4 weeks. Note the production of stearidonic acid (18:4$n-3$). 20:1 represents the sum of 20:1 isomers (20:1$n-9$ and 20:1$n-11$).

3.4. Fatty acids mass balance analysis

The results of the mass balance analysis are shown in Fig. 1. During the 2 and 4 weeks purging, there was a general reduction in total fatty acids present in the fillet. Amongst all fatty acids, only stearidonic acid (18:4$n-3$) was produced *ex novo* (2.1 and 9.7 mg in 2W and 4W, respectively).

3.5. Flavour volatile compound analysis

A total of 32 volatile compounds were identified. The majority of compounds were aldehydes (17) followed by hydrocarbons (10), alcohols (1) and others (4) (Table 5). No ketones were identified in the present study. Hexanal, nonanal, hexadecanal, 9-octadecenal, and octadecanal were the aldehydes found in highest concentrations. Hexanal was higher ($P < 0.05$) in 2W ($3.39 \pm 0.55\%$) compared to 4W, while Hexadecanal, octadecenal, nonanal, and octadecanal were higher ($P < 0.05$) in 0W ($28.14 \pm 3.19\%$, $6.58 \pm 0.67\%$, $4.63\% \pm 0.42$, $2.66 \pm 0.27\%$, respectively).

Pentadecane and 2,6,10,14-tetramethylpentadecane, a compound commonly named pristane, were the hydrocarbons found in highest concentration. Pentadecane was highest in 2W ($18.41 \pm 1.30\%$), while pristane was lowest in 0W ($23.49 \pm 2.94\%$).

Table 5
Volatile compounds (% total volatile compounds) isolated and identified in Murray cod fillet purged for 0, 2 and 4 weeks

Compound	RI	Treatment			Description
		0 weeks	2 weeks	4 weeks	
2,4-Decadienal	1204	1.94 ± 0.25^{b}	0.99 ± 0.15^{a}	1.22 ± 0.18^{a}	Green, fried fat, cod oil
2,4-Heptadienal	1002	1.72 ± 0.21	2.16 ± 0.46	2.67 ± 0.21	Green, cucumber
2,6-Nonadienal	1137	0.00 ± 0.00	0.48 ± 0.02	0.31 ± 0.05	Green, cucumber
2-Decenal	1127	0.94 ± 0.40	0.00 ± 0.00	0.58 ± 0.16	Orange, fatty fried
2-Hexenal	855	0.38 ± 0.00	0.55 ± 0.12	0.36 ± 0.09	Moss, mushroom
2-Nonenal	1141	0.65 ± 0.06	0.73 ± 0.23	0.43 ± 0.07	Orris-like, citrus
2-Pentenal	768	0.64 ± 0.07	0.66 ± 0.14	0.73 ± 0.14	Green, grass
9,17-Octadecadienal	2372	0.87 ± 0.18	0.00 ± 0.00	1.84 ± 0.56	–
9-Octadecenal	1344	6.58 ± 0.67^{b}	3.02 ± 0.85^{a}	6.05 ± 0.62^{a}	–
Decanal	1167	0.00 ± 0.00	0.42 ± 0.12	0.35 ± 0.06	Beefy, musty, cucumber
Heptanal	904	1.31 ± 0.11^{ab}	1.54 ± 0.21^{b}	0.99 ± 0.10^{a}	Oily, fatty, rancid
Hexadecanal	1321	28.14 ± 3.19^{c}	10.88 ± 2.55^{a}	19.85 ± 1.64^{b}	Cardboard
Hexanal	804	2.34 ± 0.23^{ab}	3.39 ± 0.55^{b}	1.90 ± 0.20^{a}	Herbaceous, oxidised
Nonanal	1103	4.63 ± 0.42^{b}	1.95 ± 0.20^{a}	1.58 ± 0.20^{a}	Piney, floral, citrus-like
Octadecanal	1348	2.66 ± 0.27^{b}	1.22 ± 0.27^{a}	1.83 ± 0.19^{a}	–
Octanal	1009	0.55 ± 0.08	0.00 ± 0.00	0.31 ± 0.03	Tallowy, citrus-like
2-Methyl pentanal		0.42 ± 0.24	0.79 ± 0.13	0.94 ± 0.15	–
∑Aldehydes		53.37 ± 4.80^{c}	27.24 ± 3.55^{a}	42.70 ± 1.77^{b}	
1-Pentanol	749	3.40 ± 0.19	2.93 ± 0.69	3.09 ± 0.37	–
∑Alcohols		3.40 ± 0.19	2.93 ± 0.69	3.09 ± 0.37	
1,5-Cyclooctadiene	1083	0.61 ± 0.04	0.70 ± 0.08	0.57 ± 0.05	–
1-Heptadecene	1302	0.00 ± 0.00	0.52 ± 0.04	0.34 ± 0.04	–
3,5-Octadiene	810	0.18 ± 0.01^{a}	0.60 ± 0.07^{c}	0.39 ± 0.02^{b}	–
8-Heptadecene	1298	0.61 ± 0.07^{a}	0.80 ± 0.03^{b}	0.56 ± 0.04^{a}	–
Caryophyllene		0.00 ± 0.00	0.86 ± 0.10	0.68 ± 0.08	–
Heptadecane	1303	1.79 ± 0.21^{a}	3.20 ± 0.34^{b}	1.98 ± 0.18^{a}	Alkane
Hexadecane	1285	0.00 ± 0.00	0.48 ± 0.06	0.27 ± 0.00	Alkane
Octane	800	0.83 ± 0.09^{b}	0.44 ± 0.07^{a}	0.38 ± 0.04^{a}	–
Pentadecane	1264	10.28 ± 1.54^{a}	18.41 ± 1.30^{b}	11.28 ± 0.84^{a}	Alkane
Pentadecane, 2,6,10,14-tetramethyl-	1304	23.49 ± 2.94^{a}	40.45 ± 2.51^{b}	33.67 ± 1.25^{b}	Green, sweet, crayfish
∑Hydrocarbons		37.44 ± 4.96^{a}	65.63 ± 4.37^{c}	50.24 ± 1.90^{b}	
Furan, 2-propyl		0.93 ± 0.13	1.06 ± 0.18	0.00 ± 0.00	–
1,2-Benzenedicarboxylic acid		1.95 ± 0.21	1.44 ± 0.21	1.22 ± 0.43	–
2,4-Di-tert-butylphenol		2.21 ± 0.42	2.35 ± 0.81	2.60 ± 0.55	–
3-Tert-butylphenol		1.17 ± 0.13	0.00 ± 0.00	0.16 ± 0.07	–
∑Others		5.80 ± 0.50	4.21 ± 1.03	3.98 ± 0.40	
Total $n-3$ derived aldehydes		2.65 ± 0.41	3.23 ± 0.80	3.94 ± 0.09	
Total $n-6$ derived aldehydes		4.91 ± 0.88	4.78 ± 1.04	3.90 ± 0.58	

Values are mean ± SEM.
Values in the same row with the same superscripts are not significantly different ($P > 0.05$).

Table 6
Number of correct responses (odd sample positively identified) 1 following the consumer preference test

	0 weeks	2 weeks	4 weeks
0 weeks	–	21/34 (***)	20/34 (**)
2 weeks	21/34 (***)	–	14/34 (ns)
4 weeks	20/34 (**)	14/34 (ns)	–

The values are expressed as the ratio of the positively identified differences between samples over the total number of responses.
Note: Values between brackets show the degree of significance (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; ns = $P > 0.05$) according to Meilgaard et al. (1999).

In all, total aldehydes were highest in 0W ($P < 0.05$) (53.37 ± 4.80%) and hydrocarbons highest in 2W ($P < 0.05$) (65.63 ± 4.37%). 1-Pentanol was the only alcohol found amongst the treatments.

3.6. Sensory evaluation

Consumers could easily distinguish between unpurged (0W) and purged fish (2W and 4W) ($P < 0.01$) but were unable to differentiate between 2W and 4W fish samples ($P = 0.1$) (Table 6). In general, consumers associated the 0W samples with an earthy/muddy or fishy descriptions

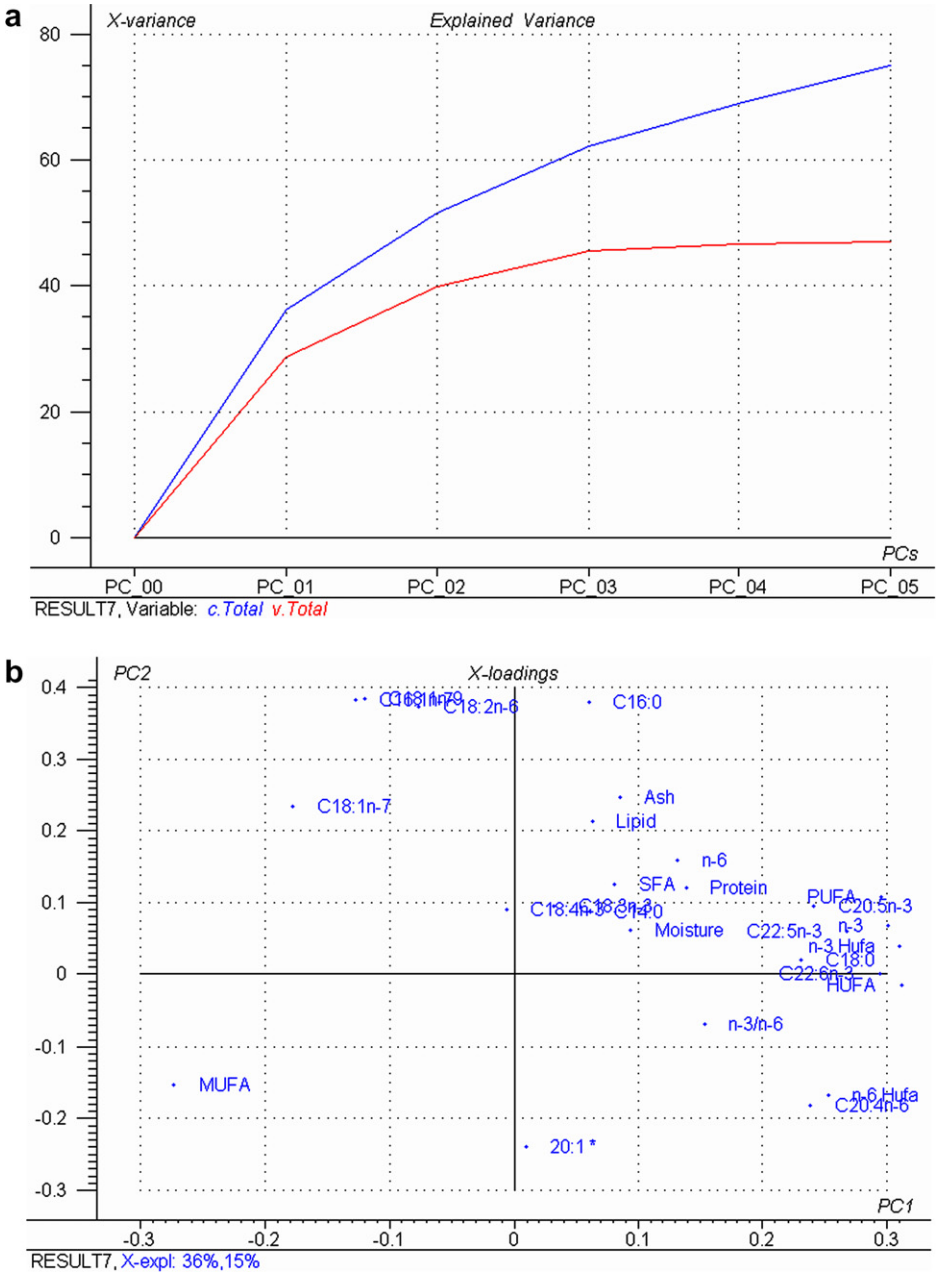

Fig. 2. (a)–(c) Explained variation, loadings and scores of the principal component analysis carried out on the proximate (%) and fatty acid composition (%) of fish purged for 0, 2 and 4 weeks.

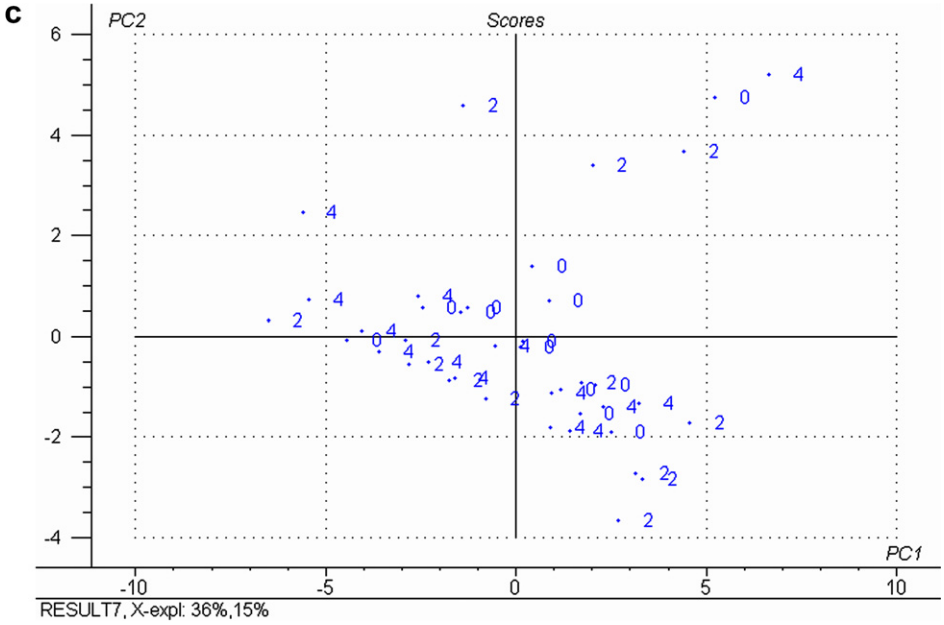

Fig. 2 (*continued*)

of flavour, and a dry texture. The 2W and 4W samples were described as sweet with a moist texture.

3.7. Principal component analysis

With the aim of exploring the weights of the variable measured, the principal component analysis was carried out on the proximate and fatty acid composition of fish. The first PC, the first two PCs and the first three PCs explained 36.2%, 51.6% and 62.1% of the total variance, respectively. In Fig. 2 the loadings and the scores plot shows the distribution of the variables and samples on the plane described by the first two PCs.

4. Discussion

As expected, multiple biological functions are altered during purging that manifest in compositional changes affecting consumer perception and liking of fish.

Purging is common practice in many aquaculture facilities to ensure that the product reaches the market with an empty gut. In more recent years, a longer term purging and associated longer starvation has also been adopted to stabilise the quality of the flesh (Einen & Thomassen, 1998). In fact, starvation can also be considered a conditioning technique as it enhances the biochemical and microbial storage stability of the carcass. By reducing the amount of faeces in the intestine, spoilage is delayed and digestive enzyme activity is reduced. If further processing steps are considered, e.g. filleting and freezing, an interruption in the feeding before slaughter may be a determinant factor of product shelf life (Huidoboro & Tejada, 2004).

Starvation is also known to affect fatty acid composition of body tissues, in particular in the liver, muscle and visceral fat deposits (De Silva, Gunasekera, & Austin, 1997). During the present study, the liver seemed to be the tissue most affected by the purging in Murray cod. Its condition, in fact, decreased with increasing purging time, indicating that Murray cod preferentially uses hepatic reserves instead of lipid from the muscle and perivisceral area, contrary to other fish species (Jezierska, Hazel, & Gerking, 1982; Regost et al., 2001). Consequently, the total perivisceral fat deposition was almost unaffected by the purging strategy.

Contrary to SFA and MUFA that remained fairly constant, PUFA, $n-3$ and $n-3$ HUFA were found at higher concentrations in purged fish. For this reason, the purging process seems to have a positive effect on the nutritional characteristics of Murray cod as previously described in different farmed species (Einen & Thomassen, 1998; Regost et al., 2001; Turchini, Mentasti, Crocco, et al., 2004). However, the recorded reduction in fatty acid, mainly due to the reduction in fillet weight and not to the reduction of total lipids, has to be taken into considerations from a consumer point of view. Hence, despite the increased $n-3$ HUFA concentration in the fatty acid make up of the fillet, the actual content of $n-3$ HUFA per fish fillet was significantly reduced in purged fish.

Although the majority of fatty acids showed a disappearance during starvation, stearidonic acid (18:4$n-3$) was the only fatty acid to be produced *ex novo*, in particular in fish purged for 4 weeks. This fatty acid is known to be produced *in vivo* by the Δ6 desaturase from α-linolenic acid (18:3$n-3$) and this is confirmed by the concurrent reduction of the latter. Accordingly, it has been documented previously that a reduction of the intake of $n-3$ PUFA can stimulate *in vivo* elongation and desaturation of C_{18} fatty acid (Turchini, Francis, & De Silva, 2006b).

Principal component analysis of proximate and fatty acid composition was unable to draw a clear picture as the experimental groupings were not perfectly distinct.

Purging, amongst other reasons, is also used to eliminate possible off flavours from tainted fish. Although preventing off flavours occurrence and best practice farm management should be the first steps to reduce the incurrence of taints in cultured fish (Lee, 2002), sometimes purging is the most convenient and effective method, both from a practical and financial point of view (Tucker, 2000). Freshwater fish are usually characterised by a sweet and delicate aroma (Turchini, Mentasti, Caprino, et al., 2004). This aroma is given by the interaction of volatile aldehydes and alcohols derived from the oxidative deterioration of $n-3$ and $n-6$ PUFA (Durnford & Shahidi, 1998; Prost, Sérot, & Demaimay, 1998; Turchini, Mentasti, Caprino, et al., 2004). The odour threshold of aldehydes is generally lower than those of other volatile compounds (Spurvey, Pan, & Shahidi, 1998), thus they have a greater contribution to total flavour. In the present study, the aldehydes concentration in unpurged fish was significantly higher than in purged fish, and this is in agreement with the consumer's responses. Surprisingly, of these aldehydes, hexanal was found in a relatively higher concentrations in fish purged for 2 weeks. This compound, which derives from the oxidation of $n-6$ PUFA and is usually considered an off flavour in seafood, can be used as an indicator of seafood and meat degradation (Shahidi & Pegg, 1994). However, in a complex matrix such as fish flesh, it may have physio-chemically interacted with other compounds, changing flavour intensity or even generating new flavours (Keast, Dalton, & Breslin, 2004). A specific compound, 2,4-di-tert-butylphenol, was found at relatively high concentrations. Skjevrak, Lund, Ormerod, and Herikstad (2005), associated this compound with the degradation process of the antioxidant Irgafos 168® which is used to protect the polymer in HDPE pipes during production and the lifetime of the pipe. Although, HDPE pipes are not part of the culture system itself, they were used to feed water from the bore, hence the possibility of water contamination.

Another compound, caryophyllene, a sesquiterpinoid constituent of many essential oils, in particular clove oil, was found in purged fish, and can attributed to the residual anesthetic (AQUI-S™) used during the preliminary stages of the trial. No traces of caryophyllene were found in unpurged fish as they were stunned culled immediately without anesthesia.

Perceived changes in flavour between 0W and the 2W and 4W samples were also detected during the consumer preference test. Consumers found it relatively easy to differentiate between unpurged and purged fish, with purging for 2 weeks sufficient to significantly increase consumer liking of Murray cod. However, increasing purging time to 4 weeks did not further increase consumer liking. Consumers found unpurged fish quite unpleasant, mainly due to a muddy/earthy off flavour. On the other hand, fish purged for 2 and 4 weeks were found rather pleasant, sweet and juicy.

Taking into consideration that consumers found a muddy/earthy flavour in unpurged fish, the presence of geosmin and 2-methylisoborneol in unpurged fish could also be a possibility. However, in freshwater aquaculture the production of these two compounds is usually associated with open pond culture, where, in conjunction with increased water temperature and light (Paerl & Tucker, 1995), cyanobacteria blooms may produce compounds that give an unpleasant taint to the cultured fish (Zimba, Grimm, & Dionigi, 2001; Zimba, Khoo, Gaunt, Brittain, & Carmichael, 2001). Seldom this has been reported in RAS (Schrader, Acuña-Rubio, Piedrahita, & Rimando, 2005), and this is confirmed by the paucity of information in the literature.

SDE has been widely used in the past for the determination of flavour volatile compounds (Cai, Liu, & Su, 2001). SDE has also been used to detect geosmin in the extracts of cooked spinach leaves (Näf & Velluz, 2000). Näf and Velluz detected an exceptionally high concentration in the extracts, second only to beetroot. In the current study, no traces of geosmin were detected in fish samples. The human sensory threshold detection limit of geosmin in fish has been reported to be between 59 and 900 ng kg^{-1}, depending on the fish species (Persson, 1980; Robertson, Hammond, Jauncey, Beveridge, & Lawton, 2006) and considering that Murray cod is characterised by a mild and delicate flavour, the detection threshold may be very low.

The results of this study aim to shed some light on the optimum purging time for intensively farmed Murray cod. Taking into consideration that fish purged for 2 weeks had a total weight loss of about 4%, an improvement in the percentage fatty acid profile and considering that they were preferred by consumers it is recommended that Murray cod should be purged for a period no longer than 2 weeks. A shorter period to the one suggested would jeopardise the marketability of the product, while a longer period would have a negative effect on the production costs. Therefore, although purging, as it is known, is essential in the quality control process, new strategies have to be studied and implemented to minimise body weight loss and maintain good edible quality.

Acknowledgments

The authors would like to thank the Victorian State Government, Department of Innovation, Industry and Regional Development, for the funding provided under the STI Project. The authors would like to thank also the VSA – Department of Veterinary Science and Technology for Food Safety, University of Milan (Italy) for the kind hospitality during the volatile compounds analyses. Finally the authors would like to thank Dave Francis for his help, prior and during the consumer test and the Australian Research Council for funding to Giovanni Turchini.

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