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Research Article

Quality of Rainbow Trout (*Oncorhynchus mykiss*) Reared in Recirculating Aquaculture System and during Depuration Based on Chemical and Sensory Analysis

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In recirculating aquaculture systems (RAS), off-flavors can accumulate in fish muscle tissue. They are problematic for consumer acceptance and the reputation of farmed fish products. Although off-flavors are not toxic at low concentrations, they often give fish muscle earthy, muddy, or other unwanted flavors. Traditionally, the study of off-flavors in fish focused on muddy and earthy off-flavors caused by geosmin (GSM) and 2-methylisoborneol (MIB), but other unwanted flavors and compounds have also been identified. In this study, the selected off-flavors were chemically quantified in fish from a RAS-rearing rainbow trout (*Oncorhynchus mykiss*) and in different stages of depuration. A group of trained panelists with experience in sensory evaluation was specifically trained in analyzing rainbow trout samples. The panelists evaluated the fish with a sensory profile of 29 sensory attributes (12 odor, 5 taste, and 12 flavor properties). Overall, the concentrations of all the studied off-flavor compounds decreased, some to below the limit of detection and others (e.g., octanal, octanoic acid, phenylacetaldehyde, and acetoin) to a certain low level. Moldy, earthy, and musty odors and flavors especially decreased during depuration compared to fish in RAS. This study shows the consistency of the chemical analysis and sensory profiling. It also provides important information about the effects of the depuration period in RAS and on the chemical and sensorial quality of rainbow trout.

1. Introduction

Freshly harvested fish has a delicate but very distinctive species-specific flavor, as reported for uncooked [1, 2], cooked [3], and smoked [4] fish products. Sensory analysis has revealed, e.g., a bitter and umami taste and a sensation of mouthfulness. The sensory properties of fish are formed by a variety of flavor-inducing volatile and nonvolatile odor compounds, which include a variety of alcohols, sulfur compounds, carbonyls, and hydrocarbons [5]. Taste sensation is also affected by peptides, amino acids, organic acids, carbohydrates, and inorganics in fish. Consumers are becoming increasingly demanding and will not compromise on the flavor or the quality of fish [6].

The sensory properties of fish vary based on fish species, their physical and chemical properties, feed ingredients, storage conditions, and processing [5, 7, 8]. During storage, some flavor compounds are transformed due to oxidation and microbial interaction into less favorable derivatives such as flat or putrid flavors. Fish is highly susceptible to oxidation and biological deterioration during storage, which emphasizes the importance of suitable storage before consumption [9].

In recent decades, land-based recirculating aquaculture systems (RAS) have become increasingly important, as they consume less water per kg of fish produced, ensure stable conditions, and are an environmentally sustainable means of meeting global food demand. However, certain unwanted flavor compounds (off-flavors) can be formed in RAS due

to microbial activity in aquaculture water and biofilms, which easily accumulate in fish muscle tissue [10]. Off-flavor compounds are typically produced as metabolic by-products of certain microbial species such as Cyanobacteria, Actinobacteria [11], *Myxobacteria*, and *Sorangium* [12]. Accumulated off-flavors are typically removed from the fish flesh by depurating the fish in clean water up to 15 days before sale [13–15]. The fish are not fed during depuration to ensure as clean water as possible, but the fish often lose weight which can lead to substantial increase in production costs [15, 16].

A wide range of compounds, including alcohols, aldehydes, and terpenes, affect the perceived sensory properties in fish. Dozens of compounds have been identified to induce off-flavors in RAS [17–19]. The most typical off-flavors perceived in fish are often described as musty and earthy, which consumers find objectionable. These flavors are typically induced by the terpene compounds geosmin (GSM, trans-1,10-dimethyl-trans-9-decalol) and 2-methylisoborneol (MIB, (1-R-exo)-1,2,7,7-tetramethyl-bicyclo [2.2.1]heptan-2-ol) [20, 21]. Typically, flavor and off-flavor compounds are volatile or semi-volatile compounds with a wide range of solubility in water, ranging from readily soluble 1,000 g L^{-1} (acetoin) to more scarcely soluble 11.8 mg L^{-1} (TCA, [22]).

Off-flavors can be perceived differently from one person to another, and the off-flavor compounds may create different sensations in various fish species [23]. Additionally, there are differences between people's abilities to sense low levels of off-flavors [24], and tasting of a large number of different compounds can be tricky. Lipid content in fish can also affect the intensity of the off-flavor sensation by increasing the sensory threshold as the lipid content increases [24]. The sensory threshold values, especially for GSM and MIB, are very low, and concentrations of 1.3–4.0 ng L^{-1} (GSM) and 6.3–15 ng L^{-1} (MIB) in water [25, 26], and 250–900 ng kg^{-1} (GSM), 700 ng kg^{-1} (MIB) in fish muscle, have been reported [27, 28] and reviewed by Lindholm-Lehto and Vielma [29]. However, for many of the off-flavor compounds, sensory threshold values in fish muscle have not been defined. Besides GSM and MIB, a variety of other off-flavor compounds can accumulate in fish muscle in RAS. Off-flavor-inducing compounds can originate from, e.g., fish feed, feed pellet production, and microbial activity in RAS and in inlet water [30, 31].

The compounds were selected for chemical analysis and sensory evaluation based on feedback and descriptions received by a commercial RAS farm rearing rainbow trout, consumer feedback, and descriptions of professional cooks. This feedback was first used in our previous study when the selected compounds were introduced to the analytical method [32], while their concentrations were quantified in this study and compared to the results of sensory evaluation.

In our previous study [32], we reported a method for analyzing 14 off-flavor compounds and their removal from fish muscle during depuration. In this study, our aim was to follow the change in sensory quality attributes of rainbow trout during the depuration period and compare the results with the content of off-flavor compounds. We hypothesized that a group of trained sensory panelists could recognize and describe the off-flavors and the change in their intensities during the depuration.

2. Materials and Methods

2.1. Experimental Setup. The experiment was conducted at the Laukaa fish farm of Natural Resources Institute Finland (Luke). Rainbow trout was reared in a pilot-scale RAS (FREA Aquaculture Solutions, Denmark). The full description of the pilot scale RAS has been reported by Pulkkinen et al. [33]. In short, the RAS consisted of two identical units, each unit with two 5 m^3 raceway rearing tanks and a 1 m^3 space for sludge cones which collect settleable solid material and uneaten feed. From the tanks, water flowed through a drum filter (60 μm mesh size, Hydrotech HDF800, Veolia, France) and two parallel 2.5 m^3 fixed bed bioreactors filled with 1.5 m^3 Saddle chips (KSK Aqua, Denmark). Finally, water flowed (water flow rate measured with Fluxus F501, Flexim, Germany) through a 2.24 m^3 degassing unit and a 0.74 m^3 pump sump and was pumped through a low-head oxygenator (FREA, Aquaculture Solutions, Denmark) back to the fish tanks. Dissolved oxygen (Oxi:lyser, s::can, Austria) was monitored online and maintained above 8.0 mg L^{-1} in the rearing tanks. The measurement data were stored on an industrial computer (Con::cube, S::can, Austria).

The water temperature was adjusted at 12.8°C by controlling the hall air temperature. The pH was maintained at 7.5 (ProMinent, Germany) by adding dissolved sodium bicarbonate to the pump sumps (EJ-R, Iwaki, Japan). Clean inlet water (Watson Marlow 630, Spirax-Sarco Engineering, UK) was led from the oligotrophic Lake Peurunka (62.44886, 25.85201, area 694 ha, 59,600 m^3). The inlet water was a 1 : 1 mixture of surface water (depth of 4 m) and the aphotic layer (depth of 8 m). Replacement water from Lake Peurunka was taken at 500 L kg feed $^{-1}$ (5.2–7.2 m^3 d^{-1}).

The total nitrogen (TAN, 0.8 mg L^{-1}), nitrite-N (0.105–0.108 mg L^{-1}), and nitrate-N (44.2–65.4 mg L^{-1}) were analyzed on-site using quick spectrophotometric tests (Procedure 8038 Nessler, LCK341/342, and LCK340 for DS 3900, Hach, USA). The water alkalinity (88.3–113.1 mg L^{-1}) was measured by a standard titration method ISO 9963-1 : 1994 (TitraLab AT1000, Hach, Loveland, USA) and turbidity (5.5–6.6 NTU) with Hach 2100q Turbidimeter (Hach, Loveland, USA).

2.2. Fish and Feeding. In total, 189 fish were reared for 3 months in RAS with an average weight of 1.18 kg and biomass of 223 kg (tank density 45 kg m^{-3}) and increased to 1.89 kg (123 individuals, a biomass of 232 kg, tank density of 46 kg m^{-3}) during the experiment. Prior to RAS, fish were reared in a partial reuse system (relative water renewal rate 4,000 L kg^{-1} feed, average weight 0.341.89 kg) and originated from the Hanka-Taimen Hatchery (Hankasalmi, Finland). Supernumerary fish were regularly removed to maintain the tank biomass and fish density at a suitable level. The fish were visually inspected on a daily basis.

The fish were fed by an automated feeding system (T Drum 2000, Arvo-Tec, Finland) with a commercial fish feed (BioMar Orbit, 6 mm) containing crude protein (37%–40%), crude lipid (31%–34%), carbohydrates

Table 1: Selected off-flavor compounds, induced aromas, solubilities in water (at 20°C), and sensory limits reported in the literature.

Compound	Aroma	Solubility in water at 20°C	Sensory limit	Media	Reference
Acetoin/3-hydroxy-butan-2-one/	Buttery	1,000 g L^{-1}	150 mg L^{-1}	Wine	[35]
Caproic acid/hexanoic acid	Goat-like	10.8 g L^{-1}	420 μg L^{-1} 1.8–3.6 mg L^{-1}	Wine, tea	[36, 37]
Caproic aldehyde/hexanal	Grass	4.49 g L^{-1}	0.3 14 μg L^{-1}	Water	[38]
Caprylic acid/octanoic acid	Fruity-acid, irritating	0.68 g L^{-1}	500 μg L^{-1} 0.16–1.9 mg L^{-1}	Wine, tea	[36, 37]
Caprylic aldehyde/octanal	Fruit-like, citrussy	560 mg L^{-1}	0.7–1.75 μg L^{-1}	Water, wine	[38]
Geosmin/dimethyl-8-hydronaphtalen	Musty	160 mg L^{-1}	2–10 ng L^{-1}, 15 ng L^{-1}, 250–900 ng kg^{-1}	Water, fish	[27, 39, 40]
3-Isobutyl-2-methoxy- pyrazine	Undesirable, musty	20.9 g L^{-1}	1–2 ng L^{-1}	Wine, water	[41, 42]
2-Isopropyl-3-methoxypyrazine	Undesirable, musty	61.4 g L^{-1}	2 ng L^{-1}	Water	[41, 42]
2-Methylisoborneol	Earthy	305 mg L^{-1}	100–700 ng kg^{-1}, 2–10 ng L^{-1}, 15 ng L^{-1}	Fish, water	[25, 39, 40]
3-(Methylthio)propion-aldehyde	Onion-like, meat-like	insoluble	0.2–0.5 μg L^{-1}	Beer, water	[43, 44]
Phenylacetaldehyde	Sweet, rose, flowery	2.21 g L^{-1}	1 μg L^{-1}	Water	[43]
α-Terpineol	Terpenic	2.4 g L^{-1}	330–350 μg L^{-1}	Water	[19]
2,4,6-Trichloroanisole	Medicinal, phenolic, iodine-like	11.8 mg L^{-1}	1–10 ng L^{-1}, 7 ng L^{-1}	Water	[42, 45]
Vanillin/4-hydroxy-3-methoxybenzaldehyde	Vanilla, sweet	10 g L^{-1}	20 μg L^{-1}	Water	[46]

(15.5%–21.5%), ash (3.3%–3.5%), and total phosphorous 0.8%, as given by the manufacturer. The feed ratio ranged from 0.6% to 0.8%, resulting in a feed conversion ratio of 1.2.

In January 2022, 48 individuals with an average weight of 1.74 kg and a total biomass of 34.1 kg (before the sampling) were randomly selected and transferred to two 600 L depuration tanks, 24 individuals per tank (60 kg m^{-3}). The Water flow rate was kept at 2.5 L min^{-1}, and the temperature at 12–14°C. The depuration tanks were aerated (JDK-S-120, Secoh, Japan) to maintain the oxygen and carbon dioxide concentrations suitable for the fish. The depuration continued for 16 days. Feed was withheld during depuration. Additionally, 12 individuals (randomly selected from the rearing tanks) were kept in depuration for 26 days, fed 0.2% per day, and were later used as a reference (REF) (assumed to contain no off-flavors).

The study followed the protocols approved by the Luke Animal Care Committee, Helsinki, Finland, and EU Directive 2010/63/EU (Council Directive 2010/63/EU [34]) for animal experiments.

2.3. Sampling. Feeding was withheld 48 hr before the sampling. Samples were taken directly from the rearing tank in RAS (12 individuals, D0) and during depuration after 4 days (9 individuals, D4), 8 days (9 individuals, D8), 12 days (9 individuals, D12), and after 16 days (9 individuals, D16). Furthermore, 12 individuals of depurated fish were used as an REF. These individuals were depurated for 26 days and were considered to represent fully depurated fish without any off-flavors from the RAS.

A sample from the inlet water was taken directly from the inlet pipe from Lake Peurunka on day 4 of the depuration. The sample was stored frozen at −22°C until the analysis. The chemical analysis was performed during the 2 following weeks. For the sensory analysis, the samples were stored no longer than 10 weeks.

At the time of sampling, the fish weighted an average of 1.8 kg (DO 1,887 ± 348 g, D4 1,690 ± 250 g, D8 1,911 ± 527 g, D12 1,753 ± 279 g, D16 1,830 ± 500 g, REF 1,813 ± 329 g). The fish were humanely euthanized, instantly gutted, fileted, and carefully washed to ensure high quality for sensory evaluation (Fillet weights: D0 712 ± 102 g, D4 573 ± 86 g, D8 661 ± 172 g, D12 634 ± 95 g, D16 685 ± 126 g, REF 707 ± 98 g). Both fillets were used for the analyses.

The fillets were vertically cut into pieces of at least 50 g (Figure S1), and 10 × 50 g pieces were taken for the sensory analysis. The tip of the tail was discarded. The pieces of fillet were packed into vacuum-sealed plastic bags and stored frozen at −22°C until the chemical analysis and sensory evaluation. Of 12 fish individuals of both D0 and REF, 8 were randomly selected, while out of 9 fish individuals of each D4, D8, D12, and D16, 6 were randomly selected.

2.4. Chemical Analysis of Off-Flavor Compounds. The selected standards were purchased as follows (Table 1, Table S1): 3-(methylthio)propionaldehyde, phenylacetaldehyde (PhenA), and α-terpineol (Alfa Aesar), methanol (≥99.8%, J.T. Baker), NaCl (98%, Merck), 3-hydroxy-butan-2-one (acetoin), 2-isobutyl-3-methoxypyrazine (IBMP), octanoic acid, hexanal, octanal, 2,4,6-trichloroanisole (TCA), and vanillin (Sigma–Aldrich), hexanoic acid (SigmaAldrich/Supelco®), 2-methylisoborneol (MIB, 1-R-exo-1,2,7,7-tetramethyl-bicyclo [2.2.1]heptan-2-ol) and geosmin (GSM, trans-1,10-dimethyl-trans-9-decalol,

TraceCERT®, Supelco®), 2-isopropyl-3-methoxy-pyrazine (IPMP), (Tokyo Chemical Industry Co.).

The off-flavor compounds were quantified using the method reported in Lindholm-Lehto [32]. Briefly, the sample extraction was performed by an automated SPME procedure (PAL3 autosampler, CTC Analytics, Switzerland) with an SPME Arrow fiber made of DVB/carbon WR/PDMS (divinylbenzene/carboxene/polydimethyl siloxane). The pretreatment included mixing, heating, adsorption, and desorption of analytes, injection into the GC port, and conditioning of the fiber.

The samples were analyzed using a GC-QQQ (7000 Series Triple Quadrupole mass spectrometer, Agilent, Santa Clara, CA, USA). It was operated with a Phenomenex Zebron ZB-5MSi (Torrance, CA, USA) capillary column (30 m × 0.25 mm × 0.25 μm) for the separation and an electron ionization ion source. The detection was performed in multiple reaction monitoring modes.

The peak areas of the internal standard and analytes were used for quantification. The levels of detection (LOD) and quantification (LOQ) have been determined for each compound and listed for aqueous (Table S2) and fish muscle samples (Table S3). The LOQs ranged from 0.1 to 0.7 ng L^{-1} for aqueous and from 15 to 107 ng kg^{-1} for fish muscle samples. No detectable concentrations of the analytes were found in the blanks. The full method description and validation have been reported by Lindholm-Lehto [32].

2.5. Sensory Evaluation. The samples were thawed overnight at 5°C. They were cooked sous vide at 55°C for 25 min prior to the sensory analysis. The cooking took place in the original vacuum packages. The samples were kept on a hotplate until evaluation (max. 15 min) to serve them warm one at a time to each panelist.

The sensory properties of the samples were analyzed using a generic descriptive method. A panel ($n = 8$) was recruited from a group of trained sensory panelists. The acuity of the panelists' senses was tested, and they had previous experience of the sensory evaluation of various food samples. The panel was specifically trained in analyzing rainbow trout samples. The descriptive analysis consisted of four 1 hr training sessions, one training evaluation, and three evaluation sessions. In the first training session, the samples were presented to the panelists, and they were asked to describe their odor, appearance, texture, mouthfeel, taste, and flavor. This was followed by a group discussion.

In the following training sessions, the verbal descriptions of the lexicon were clarified, and suitable REF samples and their intensities were defined to reflect the corresponding sensory attributes and their intensities. The final sensory profile consisted of 29 sensory attributes (12 odor, 5 taste, and 12 flavor properties). The attributes were evaluated on a scale from 0 to 10, verbally anchored at the end (0 = not at all, 10 = very strong). The attributes and REF samples are presented in Table 2.

Sensory evaluations were performed in the sensory laboratory of the Functional Foods Forum, University of Turku, Turku, Finland (ISO-8589:1988). The samples were coded with random three-digit numbers and served in randomized order on white porcelain plates. Compusense® Cloud software (Version 22.0, Compusense Inc., Guelph, ON, Canada) was used for data collection.

2.6. Statistical Analysis. A statistical analysis of the sensory evaluation was performed using IBM SPSS Statistics 28 (Armonk, NY, United States). Statistically significant differences between samples of different time points were calculated with a one-way analysis of variance test with Tukey's honestly significant difference or Tamhane's T2 post-hoc tests, depending on the equivalence of variance. The limit for the statistical significance level was set at $p < 0.05$.

Average sensory scores and chemical data were plotted with Partial Least Squares Regression (PLS-R) using Unscrambler (Version 10.5, Aspen Technology Inc. Bedford, MA, United States). The variables were weighted by dividing them by standard deviation. Full cross-validation was used.

For the statistical analysis of chemical off-flavor results, SPSS Statistics (IBM Corp.©, version 27.0.1.0) was employed for the polynomial regression analysis. A general linear regression model with a second-order polynomial was suitable at statistical significance level at $p < 0.05$.

3. Results

3.1. Chemical Analysis of Off-Flavor Compounds. In the inlet water, the concentrations of the selected off-flavor compounds ranged from below the LODs (Table S2) to 26.5 ng L^{-1} (Table 3).

In D0 (Figure 1(a)–1(g)), the highest concentrations were found for GSM (950 ng kg^{-1}), MIB (1,600 ng kg^{-1}), and octanal (600 ng kg^{-1}), while the concentrations of other off-flavor compounds remained below 250 ng kg^{-1}.

The depuration in clean water continued for 16 days. During this period, the concentrations of all the studied compounds decreased (Figure 1(a)–1(g)). The concentrations of some of the compounds decreased to below the LOD, while the others (acetoin, hexanal, octanal, octanoic acid, PhenA, and vanillin) remained at a certain low level. Even after 26 days (REF), no further decrease in concentrations was observed. Additionally, the concentrations of TCA were at a very low level (< LOD, Table S3) throughout the sampling period.

3.2. Sensory Evaluation. A total of 29 sensory attributes was identified from the rainbow trout samples. The results of the sensory evaluation can be seen in Figure 2.

D0 differed significantly from some or all of the other samples in total intensity of odor and flavor, fish-like odor and flavor, fatty odor and flavor, mud-like odor and flavor, earthy odor and flavor, moldy odor and flavor, musty odor and flavor, sea-like odor, bitter taste, and total intensity of aftertaste. The intensities of the sea-like odor, fish-like odor and flavor, and fatty odor and flavor were significantly milder in D0 than in some or all of the other samples. The rest of the sensory properties mentioned above were stronger in D0 than in some or all of the other samples. The strongest intensities of sensory properties were in D0 in the total intensity of taste (7.9), musty flavor (7.8), and total intensity of

Table 2: Selected sensory attributes (12 odor, 5 taste, and 12 flavor properties).

Sensory attribute	Description	Reference sample (intensity)
Total intensity of odor	The total intensity of all odor properties	
Grass-like odor	Odor of grass, moss, and fresh green	Cis-3-hexen-1-oli (only during training to identify the odor)
Sea-like odor	Odor of salt water, reeds, and wet green	Second cooking: water of 10 g Kombu (WISSI Atlantic vegetables) boiled twice in 1 L of clean: water for 30 min (4)
Fish-like odor	Odor of fish and fatty fish	Fish oil (Ecolomega natural) (6)
Fatty odor	Odor of oil, fatty fish, and fish oil	Fish oil (Ecolomega natural) (5)
Seaweed-like odor	Odor of algae and especially seaweed	Cooking: water of 10 g Kombu (WISSI Atlantic vegetables) boiled once in 1 L of water for 30 min (5)
Mud-like odor	The odor when one steps onto a bed of reeds on the shore, and especially the odor that arises from an oxygen-free condition. Can also be a mud-like odor, an odor of clay from city road construction works, or an odor of rotten egg, damp grass, or fodder compost	
Earthy odor	The odor of an underground cellar. The odor of cellar or soil is related to an earthy and/or musty odor	
Moldy odor	The odor associated with mold	
Musty odor	All non-fresh odors (e.g., mud-like, earthy, moldy, and other odors)	
Rancid odor	The odor of rancid and oxidized fat/oil	Butyric acid (during training to identify the odor in the sample)
Metallic odor	The odor of iron and metal	
Total intensity of taste	The total intensity of all taste and flavor properties	
Sweetness	The taste of sweetness	0.3% (w/v) sucrose (5)
Saltiness	The taste of saltiness	0.2% (w/v) NaCl (8)
Umami	The taste of umami	0.1% (w/v) sodium glutamate (7)
Sourness	The taste of sourness	0.03% (w/v) citric acid (8)
Bitterness	The taste of bitterness	0.03% (w/v) caffeine (7)
Fish-like flavor	The flavor of fish and fatty fish	Fish oil (Ecolomega natural) (7)
Fatty flavor	The flavor of oil, fatty fish, and fish oil	Fish oil (Ecolomega natural) (5)
Sea-like flavor	The flavor of salt water, reeds, and wet green	Second cooking water of 10 g Kombu (WISSI Atlantic vegetables) boiled twice in 1 L of fresh water for 30 min (5)
Seaweed-like flavor	The flavor of algae and seaweed	Cooking water of 10 g Kombu (WISSI Atlantic vegetables) boiled once in 1 L of water for 30 min (8) Second cooking water of 10 g Kombu (WISSI Atlantic vegetables) boiled twice in 1 L of fresh water for 30 min (5)
Mud-like flavor	The flavor when one steps onto a bed of reeds on the shore, and especially the odor that arises from an oxygen-free condition. Can also be a mud-like odor, an odor of clay from city road construction works, or an odor of rotten egg, damp grass, or fodder compost	
Earthy flavor	The flavor of an underground cellar. The flavor of cellar or soil is related to an earthy and/or musty flavor	
Moldy flavor	The flavor associated with mold	
Musty flavor	All non-fresh flavors (e.g., mud-like, earthy, moldy, and other flavors)	
Rancid flavor	The flavor of rancid and oxidized fat/oil	Butyric acid (by smelling, during training to identify the flavor of the sample)
Metallic flavor	The flavor of iron and metal	
Total intensity of aftertaste	The taste and flavor that stays in the mouth after swallowing	

Descriptions, and reference samples were used for the sensory evaluation. The intensities of reference samples on a scale of 0–10 are in parentheses.

Table 3: Detected concentrations of the off-flavor compounds in the inlet water from Lake Peurunka.

Compound	Inlet water
Acetoin	22.8 ± 1.2
GSM	2.7 ± 0.2
Hexanal	17.5 ± 5.5
Hexanoic acid	<LOD
IBMP	<LOD
IPMP	<LOD
MIB	6.9 ± 5.6
Methional	5.3 ± 1.8
Octanal	<LOD
Octanoic acid	26.5 ± 3.7
Phenylacetaldehyde	2.1 ± 0.3
TCA	0.1 ± 0.1
α-Terpineol	9.6 ± 2.5
Vanillin	5.7 ± 0.9

<LOD below the limit of detection. Inlet water was also used for the depuration ($ng\,L^{-1}$, ±SD, $n = 6$).

odor (7.5). Additionally, D4 differed significantly from REF in mud-like flavor and from D12, D16, and REF in earthy flavor. D4 and D8 differed significantly from D12, D16, and REF in musty flavor.

PLS-R (Figure 3) explains 84% of the variation. The first principal component (PC) describes the correlation between off-flavor properties and chemical compounds in the samples. Hexanal, methional, octanal, hexanoic acid, PhenA, IPMP, acetoin, octanoic acid, IBMP, MIB, α-terpineol, TCA, vanillin, and GSM are on the right-hand side of the PC1 and are positively correlated with the total intensity of odor and flavor, a mud-like odor and flavor, an earthy odor and flavor, a moldy odor and flavor, a musty odor and flavor, a grass-like odor, a sour and bitter taste, a total intensity of aftertaste, and slightly with a seaweed-like odor and flavor. Additionally, D0 is positively correlated with the aforementioned compounds and sensory properties. The chemical compounds and sensory properties in D0 are negatively correlated with fish-like, fatty, and sea-like odors and flavors, as well as sweet and salty tastes, which are positioned on the left-hand side of PC1. PC1 also describes the correlation of the rest of the samples, as D4 is closest to D0, whereas D8, D12, D16, and REF are closer to the left hand side. PC2 describes the positive correlation between the intensities of metallic and rancid odors and flavors and D0, D16, and especially REF compared to D4, D8, and D12.

4. Discussion

In depuration, the largest number of compounds and highest concentrations were found at the beginning of the depuration period (Figures 1(a) and 1(g)). All the detected concentrations decreased during the depuration, and for most of the compounds, the concentrations decreased to below the LODs (Figure 1, Table S3). The detected concentrations of GSM and MIB before the depuration (Figure 1(d)) can be considered typical compared to the previously reported values [47, 48]) in rainbow trout before depuration, although each RAS and its off-flavors are unique.

Low concentrations of GSM and MIB were found in water even at the end of depuration. This was probably explained by the concentrations found in the inlet water (Table 3), as the depuration period was run in flow-through mode, and the water was led directly into the depuration tank.

The concentrations of GSM and MIB remained below their sensory threshold values in water (below $15\,ng\,L^{-1}$ [24, 25, 27]) in the inlet and in the depuration water. The low concentrations of the compounds in the inlet water allowed the concentrations in fish to decrease to low level during depuration (Figure 1(d)).

IPMP and IBMP may originate from the thermal treatment in feed pellet formation [22, 49]. This suggests that IBMP would have accumulated in the fish muscle via the feed and intestinal tract before the depuration period. The feed was withheld during depuration, and the concentrations of IPMP and IBMP decreased to below the LODs after 16 days (Figure 1(c), Table S3).

Many of the off-flavor compounds detected in fish are produced by microbial metabolism, such as Streptomyces and cyanobacteria, especially during warmer times of year [10]. For example, TCA is a methylation product of trichlorophenols and is produced by certain microbial strains (*Penicillium*, *Aspergillus*, *Actinomyces*, *and Streptomyces*), many of which can also produce GSM and MIB [11, 12]. Small concentrations of off-flavor compounds are often found in the surface water because microbial metabolism is known to increase during spring and summer [30, 31]. It is, therefore, not uncommon for low concentrations to be detected in the inlet water. This study was performed in the winter of 2022, and the overall concentrations were very low in the inlet water. The concentrations detected here were lower than those reported in our previous study [32], probably due to seasonal variations (June–July 2021 [15] and January 2022 in this study).

The off-flavor and odor properties recognized in this study were described as mud-like, earthy, moldy, and musty odor and flavor. The samples' sensory profile was also described as, e.g., sea-like, fish-like, fatty odor and flavor. The sensory profile of rainbow trout has previously been reported as sweet, sour, fish oil-like, metallic, algae-like, mushroom-like, cooked potato-like, and warm milk-like [50, 51]. Mushroom-like, potato-like, and warm milk-like sensory properties were not recognized by the panel in this study. Their absence in the samples may be because of the off-flavors and odors being stronger in intensity and more distinguishable than some of the typical sensory properties of rainbow trout.

The intensities of fish-like odor and flavor and fatty flavor increased during the depuration (Figure 2). This may be caused by the concentrations of off-flavor compounds decreasing and allowing the fish-like odor and flavor and fatty flavor to become more distinguishable in the samples. A PLS-R plot (Figure 3) also showed these sensory properties, as well as sea-like odor

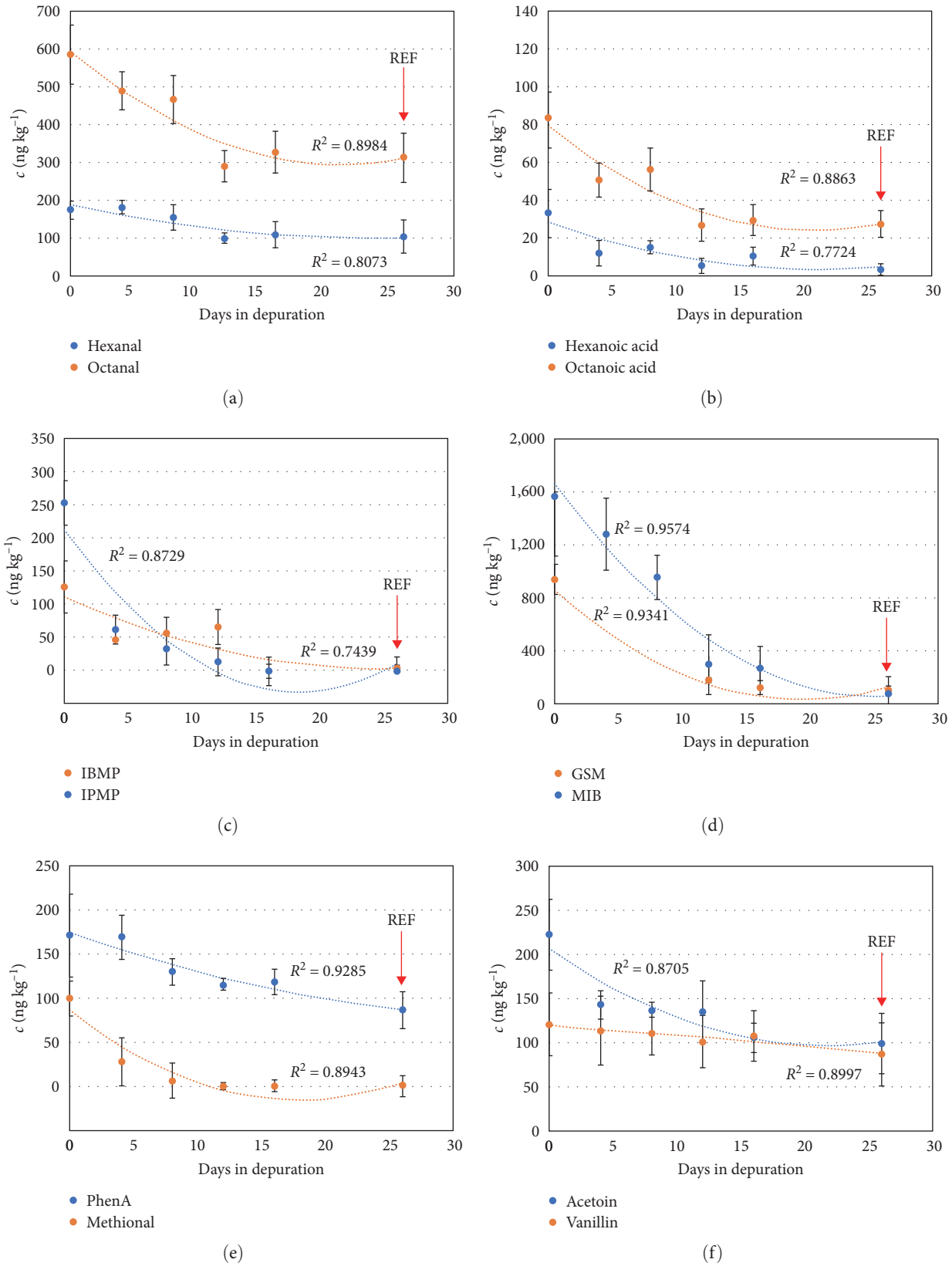
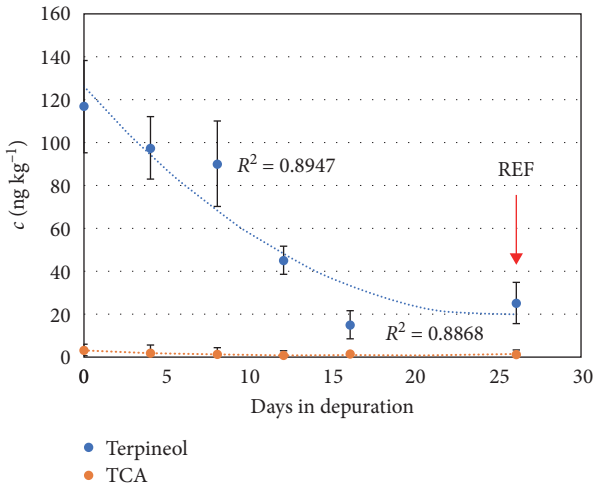
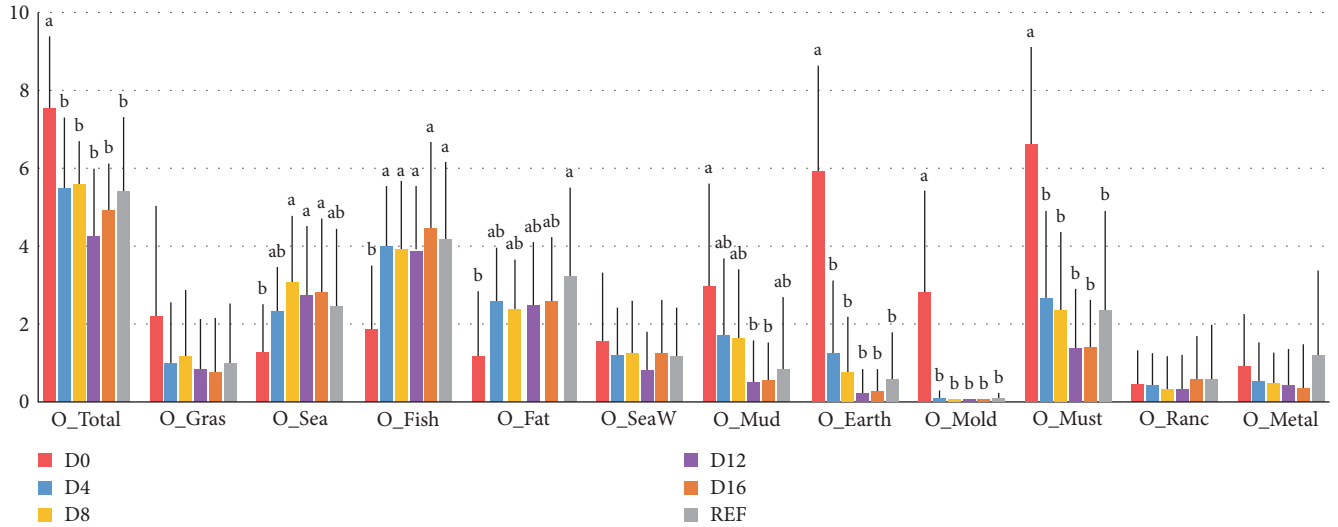


Figure 1: Continued.

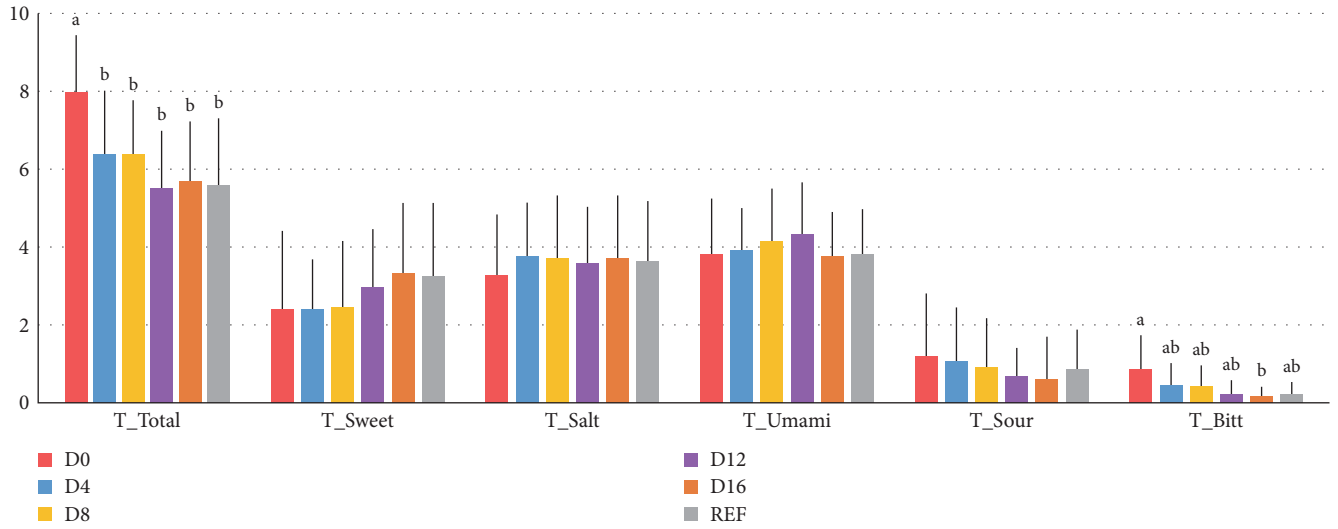


(g)

Figure 1: Concentrations of off-flavor compounds in fish muscle tissue in RAS before the depuration (day 0, ng kg^{-1}, ±SD, $n = 8$), days in depuration (days 1–16, ng kg^{-1}, ±SD, $n = 6$), day 26 used as reference (REF, ng kg^{-1}, ±SD, $n = 8$), fitted in 2nd-degree polynomial. hexanal, octanal (a); hexenoic acid, octanoic acid (b); IBMP, 3-isobutyl-2-methoxypyrazine; IPMP, 3-isopropyl-2-methoxypyrazine (c); GSM, geosmin; MIB, 2-methylisoborneol; (d); PhenA, phenylacetaldehyde, methional (e); acetoin, vanillin (f); α-terpineol, TCA, 2,4,6-trichloroanisole (g).

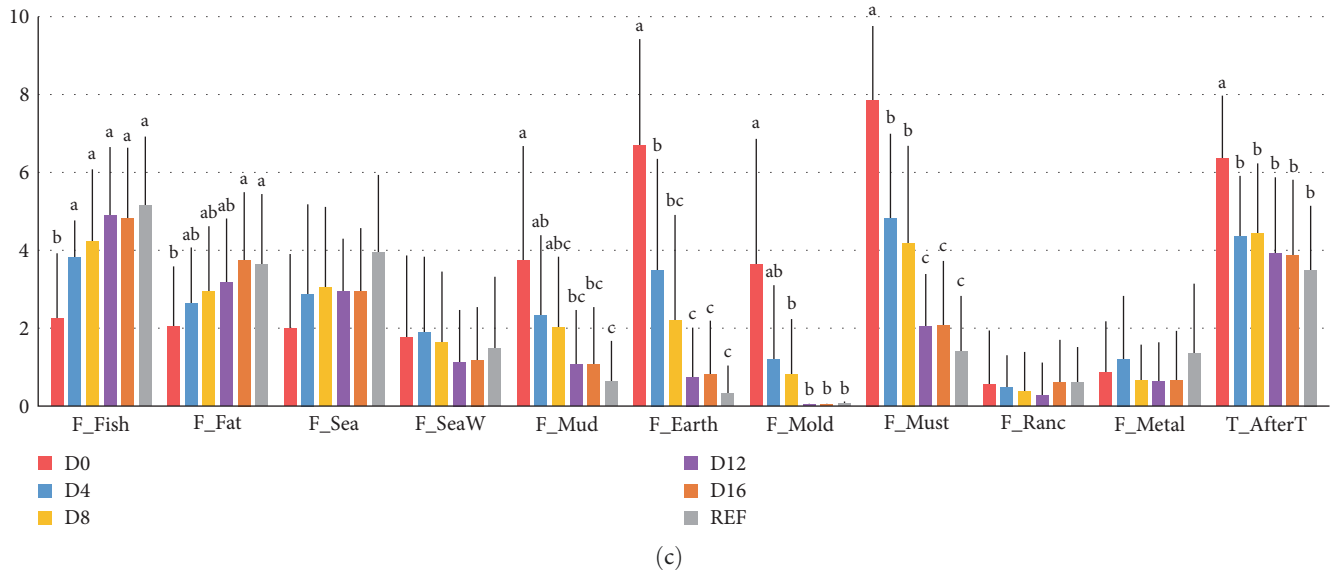


(a)



(b)

Figure 2: Continued.



(c)

FIGURE 2: The means and standard deviations of the odor (a), taste (b), and flavor properties (c) in the rainbow trout samples from different stages of depuration ($n = 24$). REF: reference, 26 days of depuration; D0–16, 0–16 days of depuration; O, odor; T, taste; F, flavor; Total, total intensity; Gras, grass-like; Sea, sea-like; Fish, fish-like; Fat, fatty; SeaW, seaweed-like; Mud, mud-like; Earth, earthy; Mold, moldy; Must, musty; Ranc, rancid; Metal, metallic; Salt, salty; Bitt, bitter; AfterT, total intensity of aftertaste. [a,b,c] The different letters within each sensory property indicate a statistically significant difference between the samples at a 95% confidence level ($p < 0.05$).

and flavor being strongly negatively correlated with D0 and positively with D12, D16, and REF. The intensities of rancid and metallic odor and flavor did not increase significantly during the depuration, but they were most strongly correlated with REF (Figure 3). None of the analyzed compounds in this study is known to induce rancid or metallic sensations (Table 1), which suggests these odors and flavors are caused by other compounds.

The intensities of mud-like, earthy, and musty odor and flavor already began to decrease in the early stages of depuration but decreased to low intensities between days 8 and 12 of depuration at the latest (Figures 2(a) and 2(c)). The decrease can also be seen in the PLS-R plot (Figure 3), as all the compounds are clustered on the right-hand side, correlating positively with sample D0, whereas D4, D8, and D12 are chronologically more on the left-hand side.

These sensory properties are produced by GSM, MIB, IPMP, and IBMP. The concentrations of MIB and GSM in D0 were higher than the sensory threshold value in fish [28, 40]. Their concentrations decreased below the threshold values by the end of the depuration period.

The intensities of mud-like, earthy, and musty odor and flavor decreased during the depuration. They seemed to start slightly increasing between D16 and REF. As MIB and GSM are lipophilic compounds, they accumulate in the lipid tissue of fish. The lipid content of fish affects the perception of these off-flavor compounds by increasing the sensory threshold values [24]. The lipid content of fish decreased during the depuration because the feed was withheld [52]. The intensities of the compounds are thus likely to increase. However, no statistically significant differences were detected between D16 and REF in any sensory property.

Besides GSM and MIB, other off-flavor compounds in fish muscle have been studied only rarely. Previously, Podduturi et al. [19] studied terpenes in pangasius (*Pangasianodon hypophthalmus*) and tilapia (*Oreochromis niloticus*). They found α-terpineol in similar or higher concentrations in pangasius and tilapia than in the concentrations in rainbow trout during and after the depuration in this study. Overall, the sensory threshold limit values in fish muscle have only scarcely been available for the off-flavor compounds [28, 40].

Moderate concentrations of IPMP and IBMP were found in the fish muscle of D0, but they decreased to below the LODs after 16 days (Figure 1(c), Table S3). Very low sensory threshold values have been reported for IPMP and IBMP in wine [41, 53] and in water [42]. Some of the remaining mud-like, earthy, and musty odor and flavor may be due to IPMP and IBMP in addition to MIB and GSM.

PhenA, vanillin, and octanal, which were detected in the samples, produce sweet flowery, fruity, and vanilla-like sensations. The sensations may not be considered off-flavors in general, but they are not typically a part of the normal flavor profile of fish [22, 54]. However, the sensory panel did not recognize these sensory properties in the samples. The sensory threshold values of PhenA, vanillin, and octanal in fish have not been reported but are relatively high in other matrices (Table 1). Relatively high sensory threshold values have also been reported for hexanoic acid and for octanoic acid in tea, and acetoin in wine (Table 1). In this study, the concentrations of these compounds in the samples were relatively low and were possibly too low or were masked by other, more intense, and distinctive off-flavor properties to have been sensed by the panelists.

The odor and flavor of TCA were typically perceived as medical, phenolic, or iodine-like [45]. Although the sensory

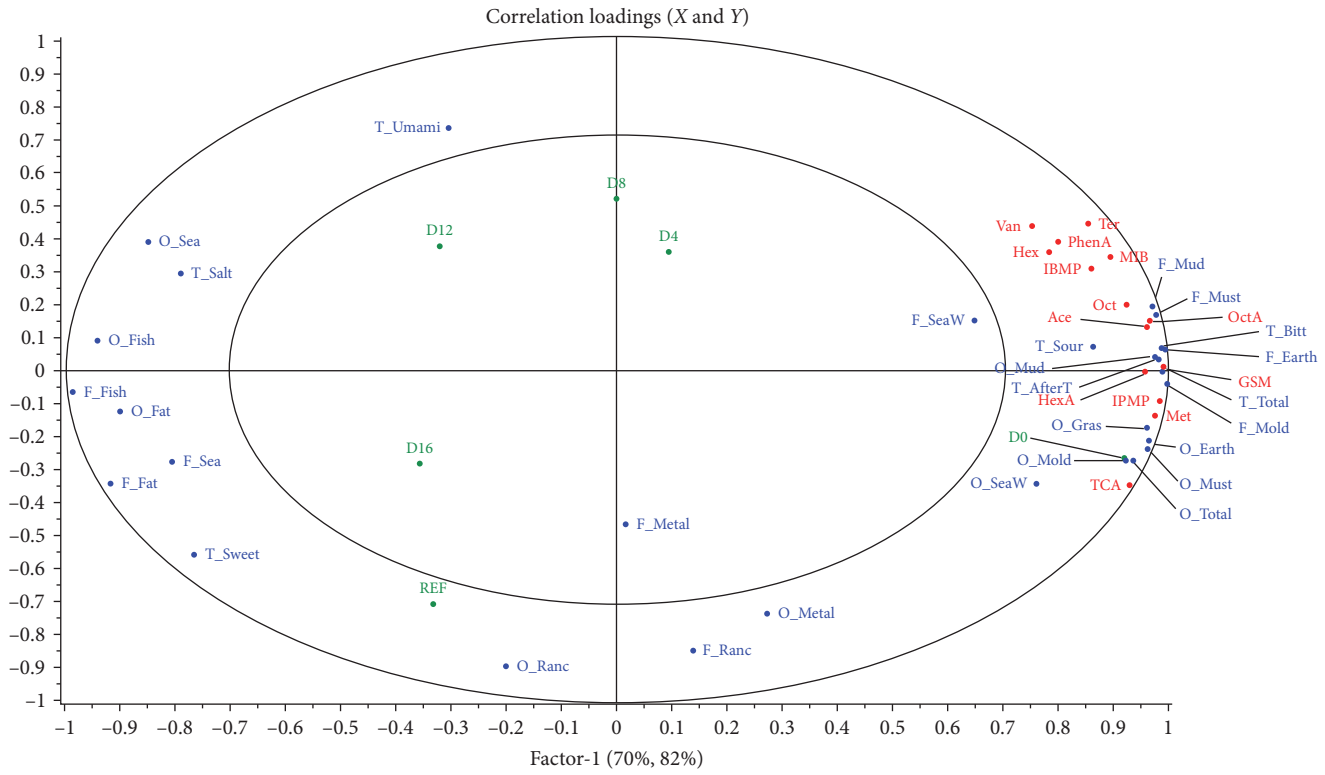


FIGURE 3: PLS-R correlation loadings plot of the sensorial properties and off-flavor compound concentrations. REF, reference, 26 days of depuration; D0–16, 0–16 days of depuration; O, odor; T, taste; F, flavor; Total, total intensity; Gras, grass-like; Sea, sea-like; Fish, fish-like; Fat, fatty; SeaW, seaweed-like; Mud, mud-like; Earth, earthy; Mold, moldy; Must, musty; Ranc, rancid; Metal, metallic; Salt, salty; Bitt, bitter; AfterT, total intensity of aftertaste. Hex, hexanal; Met, methional; Oct, octanal; HexA, hexanoic acid; PhenA, phenylacetaldehyde; IPMP, 3-isopropyl-2-methoxypyrazine; Ace, acetoin; OctA, octanoic acid; IBMP, 3-isobutyl-2-methoxypyrazine; MIB, 2-methylisoborneol; Ter, α-terpineol; TCA, 2,4,6-trichloroanisole; Van, vanillin; GSM, geosmin.

threshold value for TCA is very low [42, 45], this type of odor and flavor was not detected in the samples by the panelists. TCA has better solubility in oil than in water [42], which suggests a greater tendency to accumulate in the fish muscle.

This study combines the results of sensory analysis by trained panelists and chemical analysis. It gives valuable information on which compounds lead to sensations considered as off-flavors and -odors. These findings can help fish producers to assess and improve their depuration procedure and offer high-quality fish for consumers. However, further studies are required in order to determine the concentrations of the off-flavor compounds in fish that are accepted by consumers. Every RAS is unique, and the depuration time depends on many factors, such as the original off-flavor concentrations, quality of depuration water, lipid content in fish, and water temperature [24]. Therefore, these results do not allow a recommendation of a certain depuration time, but suitable depuration time should be determined case by case. Besides sensory evaluation, producers could benefit from using chemical analyses more extensively for the evaluation of off-flavors and sufficient depuration time.

5. Conclusions

Off-flavors in rainbow trout were studied by chemical analysis (14 selected compounds) and sensory profiling by trained panelists (29 identified sensory properties). The fish samples were taken from a RAS and at four points of a depuration period. REFs were depurated for 26 days, which is a very long time to guarantee a fully depurated sample. The concentrations of all the selected compounds decreased during depuration, some to below the LOD, and others (acetoin, hexanal, octanal, octanoic acid, PhenA, α-terpineol, and vanillin) to low levels. This is probably explained by the low concentrations of these compounds found in the inlet water.

The total intensities of odor and flavor, and typical off-flavors, such as mud-like, earthy, moldy, and musty odor and flavor, were strongest in D0. However, not all the odors and flavors induced by the studied compounds were observed by the panelists, possibly due to the masking of other compounds or higher sensory threshold limits than the concentrations in the samples. The intensities of the typical sensory properties of rainbow trout, such as sea-like and fish-like odor and flavor, as well as fatty odor, increased during the depuration.

This study showed the consistency between sensory observations and chemical analyses. Intense earthy, mud-like, and musty odors and flavors were observed, probably caused by GSM, IBMP, IPMP, and MIB. They may have also masked some of the more delicate flavors and odors, and more research is required to determine the sensory threshold limits of these compounds, specifically in fish. However,

sensory evaluation can be used in assessing off-flavor problems and anticipating consumer acceptance of cultivated fish. This study gives important information about the effect of the depuration period in RAS on the chemical and sensorial quality of rainbow trout, and producers could benefit from using chemical and sensory analyses more extensively for the evaluation of off-flavors and sufficient depuration time. However, further research is required to determine the maximum off-flavor compound concentrations regarding consumer acceptance.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical Approval

Ethical approval for the sensory evaluations was obtained from the ethical committee of the Hospital District of Southwest Finland (Sandell 145/1801/2014).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Petra Lindholm-Lehto contributed to the data curation, formal analysis, investigation, methodology, validation, writing—original draft preparation; Nora Logren contributed to the formal analysis, methodology, writing—original draft preparation; Saila Mattila contributed to the data curation, formal analysis, investigation, validation, writing—original draft preparation; Jani Pulkkinen contributed to the conceptualization, investigation, resources, writing—review and editing; Jouni Vielma contributed to the conceptualization, writing—review and editing; Anu Hopia contributed to the conceptualization, supervision, writing—review and editing.

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Supplementary Materials

Table S1: Selected off-flavor compounds, standard purity, chemical formulas, CAS numbers, densities, and aroma descriptions. Table S2: Limits of detection (LOD)s, quantification (LOQ)s, and linearities (R^2) of the selected off-flavors in the aqueous sample (1–100 ng L^{-1}) analyzed with automated SPME-GC-QQQ. Table S3: LODs, LOQs, and linearities (R^2) of selected off-flavors in fish muscle (100–1,000 ng kg^{-1}) analyzed with automated SPME-GC-QQQ. Figure S1: Fish fillet was divided into five pieces of similar size (minimum 50 g), and the tip of the tail was discarded. The pieces were used for sensory evaluation by the sensory panelists and for chemical analysis. (*Supplementary Materials*)

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