

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Inclusion of *Hermetia illucens* larvae meal on rainbow trout (*Oncorhynchus mykiss*) feed: effect on sensory profile according to static and dynamic evaluations

This is a pre print version of the following article:

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1633029> since 2019-12-24T12:51:00Z

Published version:

DOI:10.1002/jsfa.8191

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

25 **ABSTRACT**

26 BACKGROUND: Diet implementation with insect meal arouses increased
27 attention in aquaculture considering the advantages of this new protein source. The
28 effect of *Hermetia illucens* meal (HI) inclusion in diets on rainbow trout physical-
29 chemical and sensory properties was evaluated. Three diets were prepared: HI0, HI25,
30 HI50, with 0, 25 and 50% of HI replacing fish meal, respectively. Fillet sensory profiles
31 were described by descriptive analysis (DA) and Temporal Dominance of Sensation
32 (TDS) methods. Cooking Loss, WB-Shear Force, proximate analysis, fatty acid
33 composition were also determined.

34 RESULTS: Diets significantly affected fillets sensory profile. DA indicated
35 significant changes in perceived intensity of aroma, flavour and texture descriptors as a
36 function of diet composition. TDS evaluations provided information on dominance and
37 evolution of sensations perceived in fillets from different diets. The first sensations
38 perceived as dominant were related to texture attributes, followed by flavours.
39 Dominance of fibrousnesses decreased with the increasing of HI in diet. Boiled fish,
40 algae flavours and umami taste clearly dominated the HI0 dynamic profile. The onset of
41 metallic flavour dominance characterized HI25 and HI50. No differences in physical
42 parameters were detected. Principal component analysis highlighted the relationship
43 between sensory attributes and physico-chemical parameters.

44 CONCLUSION: Sensory description of fillets indicated that HI inclusion
45 induces significant differences in the perceived profile.

46

47 **Key words**

48 *Hermetia illucens*, insect feeding, rainbow trout, Descriptive Analysis, Temporal
49 Dominance of Sensation.

50

51 INTRODUCTION

52 The rising demand and consumption for aquaculture feeds have generated a
53 rapid decline in the availability of fish meal (FM) and a concurrent price increase.¹ FM
54 is the optimal animal protein source used in commercial fish feeds.² However, the use
55 of FM is both environmentally and economically unsustainable.¹ Alternative protein
56 sources have been investigated to replace FM in livestock feeds, especially in
57 aquaculture. Nowadays, insects are being considered as a novel protein source both for
58 humans and livestock.³⁻⁵ Insects grow and reproduce easily, have high feed conversion
59 efficiency, can be grown on low quality organic waste, do not compete with humans
60 and other farmed animals for nutrients and are particularly suitable for many freshwater
61 and marine fish feeding as a part of their natural diet.⁶ Moreover, they are a rich source
62 of protein, lipids, minerals and vitamins.⁷ Different insect species have been considered
63 for their possible use in fish feeds and some studies have been carried out.⁸⁻¹⁰ Among
64 the different species, *Hermetia illucens* seems to be very interesting as sustainable
65 alternative to replace FM in aquaculture feeds.⁸ *H. illucens*, is suitable to be reared on
66 organic wastes by converting them into protein-rich and lipid-rich biomass, therefore it
67 can be used for various purposes including animal feeding, biodiesel and chitin
68 production.⁶

69 Changes in fish diet affect fish flesh sensory characteristics^{11,12} such as
70 texture^{13,14} and volatile compounds.^{15,16} Previous studies showed that the replacement of
71 FM with insect protein in aqua feeds determines changes of chemical composition of
72 fish muscle, especially for lipid content and fatty acid profile.^{17,18} In several studies
73 relationships were found between fish flavour, muscle chemical composition¹⁹ and fatty
74 acid profiles.²⁰⁻²² Considering the growing interest of *H. illucens* as alternative protein

75 source to replace FM in the fish feeds, it can be highlighted that until now the studies on
76 the related effects on sensory properties of fish meat are scarce.^{17,18,23}

77 Modifying feeding practice without taking into account possible changes in fish
78 sensory properties appears a risky option²⁴, since modifications on fish flavour and
79 aroma can affect the perceived quality²⁵ and the acceptability by consumers.¹⁶

80 Descriptive Analysis (DA) and Temporal Dominance of Sensations (TDS) have
81 been found as methods providing complementary information to describe food sensory
82 properties.²⁶ DA permits identification, quantification, and description of food sensory
83 attributes.²⁷ It is useful when a detailed description of the sensory properties is desired
84 and provides a picture of the perceived differences among products in terms of intensity
85 of sensory attributes.

86 TDS tracks the evolution of “dominant” sensations (the ones catching the attention)
87 during the product evaluation.²⁸ The dynamic of perception has important consequences
88 for a better understanding of the processes used by consumers to assess acceptability
89 and sensory properties of food products.²⁹

90 In this work, DA and TDS were utilised to investigate the effect of replacing part
91 of diet proteins with *H. illucens* on sensory properties of rainbow trout (*Oncorhynchus*
92 *mykiss* Walbaum, 1792). The physical-chemical characteristics and sensory properties
93 of rainbow trout fed regular diet with FM as exclusive source of protein or fed diet
94 including *H. illucens* in partial replacement of FM were described and compared.
95 Moreover, the relationships between physico-chemical characteristics and sensory
96 profile of fillet samples were investigated.

97

98 **MATERIALS AND METHODS**

99 Sample characteristics

100 Diet formulation

101 Three isoproteic and isolipidic diets were formulated (Table 1), in which
102 *Hermetia illucens* prepupae meal (HI; Hermetia Deutschland GmbH & Co. KG
103 (HDKG), Baruth/Mark, Germany) substituted 0% (HI0), 25% (HI25) and 50% (HI50)
104 of FM

105

106 Fish feeding and sampling

107 The experimental trial was performed at the experimental facility of the
108 Department of Agricultural, Forest, and Food Sciences (Italy). A total of 360 rainbow
109 trout (*Oncorhynchus mykiss*), with an average initial weight of 178.9 ± 9.81 g, was
110 individually weighed and randomly distributed in twelve fiberglass tanks (1 m³; T:
111 $13 \pm 1^\circ\text{C}$; dissolved oxygen: 7.6 - 8.7 mg L⁻¹; water flow: 8 L min⁻¹), in an indoor
112 openwater system (30 fish per tank). Experimental diets were randomly assigned to the
113 tanks.

114 Fish were fed by hand twice a day at 1.5% of body weight, and fish were
115 weighted in bulk every 15 days to adjust the feeding rate.

116 At the end of the trial (92 days), fish were individually weighed and 30 fish for
117 each diet were slaughtered, accurately packaged inside polystyrene boxes with ice
118 covering, and brought to the Department of Agri-Food Production and Environmental
119 Sciences (Florence, Italy). After the arrival, all the fish were weighed, dissected and
120 filleted; the fillets were vacuum packaged, and frozen at -80°C until analyses.

121

122 Sample selection and preparation for sensory evaluations

123 Fish were ranked within each diet according to their weight. Individuals with the
124 highest weights within the diets and with comparable weights across diets were selected
125 for sensory evaluations (Table 2). In total, 15 individuals were selected from each diet,

126 four were utilised for Descriptive Analysis (DA) and three were utilised for Temporal
127 Dominance of Sensation (TDS) analysis. The remaining individuals were used to set up
128 the evaluation conditions and for sensory panel training purpose.

129 Before evaluations, fillets were thawed at 4°C for 24 h, washed and accurately
130 dried with paper, and skinned. The part close to tail was discharged and the ventral fish
131 bones removed. Samples were prepared by cutting the cleaned fillets in several portions
132 of 4±0.2 g each, and around 22 portions from each individual were obtained. Each
133 portion was wrapped in an aluminium foil, and stored at 6-8°C until the evaluation
134 session started.

135 Wrapped samples were steam cooked for approximately 1.30 min, until reaching
136 a temperature of 62°C at the heart and immediately presented to subjects for evaluation.

137

138 General sensory evaluation conditions

139 Samples were presented monadically and identified by a three-digit code. The
140 presentation order was randomized between subjects and sessions. The order of
141 attributes was balanced between subjects to minimize a possible “proximity” effect and
142 was always the same for a given assessor. After each sample, subjects rinsed their
143 mouth with water for 30 s, had some plain crackers for 30 s and finally rinsed their
144 mouth with water for a further 30 s. Subjects took a fifteen min break after every
145 session. Data were collected with the software Fizz (ver. 2.47.B, Biosystemes,
146 Couternon, France).

147

148 Subjects of the sensory evaluation

149 Ten subjects, 8 males and 2 females, aged from 20 to 30 years, regular fish
150 consumers, were recruited. The subjects were informed that the aim of the evaluation
151 was the description of sensory properties of fish fed diets containing also proteins from

152 insects. Before starting with the experiment, a written informed consent was obtained
153 from each subject. The subjects had no history of disorders in oral perception.

154

155 Sensory evaluation by Descriptive Analysis

156 Training sessions: sensory vocabulary development and subject training

157 Panellists participated in three training sessions of about 60 min each. The
158 subjects developed a vocabulary describing differences and similarities between
159 experimental samples in two different sessions, according to a simplified version of the
160 repertory grid method.³⁰ The initial list of attributes was reduced to achieve a list that
161 comprehensively and accurately described the product space: redundant and/or less-
162 cited terms were grouped on a semantic basis and/or eliminated according to the
163 subjects' consensual decisions. A main list of attributes was developed (Table 3) which
164 described the texture, taste and flavour of fish samples. Some standards were prepared,
165 as reported in Table 4, to induce a weak/moderate intensity. A nine point category scale
166 labelled at the extremes with "extremely weak" (corresponding to 1) and "extremely
167 strong" (corresponding to 9) was utilised for evaluation. Two repetitions of the whole
168 set of samples were performed in individual booths. Assessor and panel performance
169 were validated by evaluating two sets of samples used for the study. Panel and assessors
170 data were analysed using Panel Check software (ver. 1.4.0, Nofima, Trømso, Norway).

171

172 Evaluation

173 The evaluation of fish meat from each diet was replicated four times in four
174 sessions. In each session, each panellist evaluated three individuals, one from each diet.
175 Two samples from each individual were tested. The first sample was utilised for aroma
176 (ortho-nasal odour) and texture assessment, while the second sample was utilised for

177 taste and flavour evaluation. The overall aroma and flavour descriptors were always
178 presented as the last attribute of the relevant list.

179

180 Sensory evaluation by Temporal Dominance of Sensations

181 Subjects participated in three training sessions. In the first session, the concepts
182 of dominance and temporal evolution of sensations were explained to the subjects.
183 Then, the most relevant attributes for describing the temporal evolution of sensory
184 properties were selected from the attribute lists used for DA. Nine attributes were
185 selected: Melt in mouth, Tenderness, Juiciness, Fibrousness, Metallic, Boiled fish and
186 Algae flavours, Umami, and Astringency. Two sessions were performed for training
187 subjects with the use of the computer system for TDS data acquisition. Panellists were
188 trained to click on the “Start” button as soon as the sample was in the mouth and to
189 immediately start the evaluation. Performance of panellists and eventual artefacts were
190 evaluated by visual inspection of individual out-put of training session evaluations.

191 Panellists participated in six evaluation sessions. Two sessions per day were
192 performed and three individuals, one from each diet, were evaluated twice in the same
193 day, in two independent sessions. In total, three individuals per each diet were
194 evaluated. The total evaluation time was 90 sec.

195 Sample presentation and evaluation conditions were the same described for DA
196 evaluations.

197

198 Physical analyses

199 A number of 4 fish for each diet was randomly weighed and slaughtered.
200 Analyses on physical and chemical properties were performed on the cooked fillets of
201 each sample. The cooking loss (CL) was calculated by measuring the difference in
202 weight of the fillet before the cooking process and after, according to the formula:

203 $100 \times [\text{raw fillet weight} - \text{cooked fillet weight (g)} / \text{raw fillet weight (g)}]$

204

205 Texture analyses were carried out using a Zwick Roell[®] 109 texturometer (Ulm,
206 Germany) with the Text Expert II software, equipped with a 1 kN load cell. The
207 Warner-Bratzler shear force test (WB-SF) was performed on the cranial part of the fillet
208 epaxial region (two measurement for each fillet). A straight blade (width of 7 cm),
209 perpendicular to muscle fibre direction, was utilised at a crosshead speed of 30 mm/min
210 to 50% of total deformation. Maximum shear force, defined as maximum resistance of
211 the sample to shearing³¹ was determined from the plot of force (N) compared with
212 deformation (%) and expressed as mean.

213

214 Chemical analyses

215 Proximate composition of HI meal, experimental diets and cooked freeze-dried
216 fillets from the three groups of fish differently fed was determined according to AOAC
217 procedures.³² Dry matter, ash, crude protein and ether extract were determined
218 according to 950.46, 920.153, 976.05, and 991.36 methods, respectively.

219 The fatty acid (FA) group composition was analysed on total lipid extract³³ of HI
220 meal, experimental diets samples and cooked muscle samples obtained from fish fed
221 different diets. The FAs composition was determined by gas chromatography (Varian
222 GC 430; Agilent, Palo Alto, CA, USA) equipped with a flame ionization detector and a
223 Supelco Omegawax[™] 320 capillary column (30 m \times 0.32 mm i.d., 0.25 μ m film and
224 polyethylene glycol bonded phase; Supelco, Bellefonte, PA, USA). FAs were identified
225 by comparing the FAME retention time with the standard Supelco 37 component
226 FAME mix (Supelco). Individual FAs were quantified using tricosanoic acid (C23:0)
227 (Supelco) as internal standard. FAs were expressed as a percentage of total FAME.

228

229 Statistical analysis

230 Intensity data from the trained panel were analysed by multi-block PCA
231 (Tucker-1) and by P^* MSE plot (Panel Check software, ver. 1.4.0, Nofima, Norway) to
232 assess panel calibration and assessor performance, respectively.³⁴ Based on the P^* MSE
233 plots and Tucker-1 plot, 2 out of 10 subjects were considered unreliable and were taken
234 out from further data analysis.

235 Principal Component Analysis (PCA) was carried using the mean data for each
236 repetition, in order to geometrically represent the variability associated to diets and
237 individuals by using The UnscramblerX 10.3 software (Norway). Samples were
238 included as dummy variables (downweighted in the data matrix) to improve the visual
239 interpretation.³⁵ The full cross validation was computed to validate the interpretation of
240 the first two components.

241 Every sensory attribute was analysed following a two-way factorial design in
242 which the diets and panellist were treated as a fixed effect and as a random variable,
243 respectively.

244 The mean dominance curves for each treatments and six repetitions were
245 computed from raw software coding (1 selected; 0 not selected). The data of TDS were
246 analysed by the software Fizz (ver. 2.47.B, Biosystèmes, Couternon, France). When the
247 TDS curves were plotted, two additional lines were drawn for the chance and significance
248 levels. The chance level refers to the dominance rate that an attribute could obtain by
249 chance. Its value is inversely proportional to the number of attributes ($P_0=1/p$, where p is
250 the number of attributes). The significance level (P_s) is the minimum value this
251 proportion should be equal if it is to be considered significantly ($p<0.05$) higher than P_0 .
252 Rosner³⁶ recommended that $np(1-p)>5$ (where n =number of trials and p =probability of
253 success). In the present study, 10 panellists performed six replications of each product and
254 nine attributes were utilised, thus the number of observation was satisfied ($np= 5.87$).

255 Normality of data distributions was tested by the Kolmogorov-Smirnov test on
256 cooking loss, WB-shear force, proximate analysis and fatty acid composition. One-way
257 ANOVA was performed on physical-chemical results, considering the diets as main
258 effect. The Bonferroni post-hoc test was applied to check the significance of the
259 differences among diets, using SPSS version 17.0 software (SPSS Inc. Illinois, USA).

260 A PCA was calculated after standardization of variables in order to assess the
261 relationship among the sensory, physical and chemical dataset, using The UnscramblerX
262 10.3 software (Norway).

263

264 **RESULTS**

265 Descriptive analysis

266 The analysis of principal components (PCA) was performed using all tested
267 individual as independent samples, in order to evaluate the differences due to both the
268 different diets and those relevant to biological variability among of individuals reared
269 following the same diet. The PCA correlation loading plot (Figure 1) showed that
270 samples were mainly discriminated along the first component (PC1: 42% explained
271 variance) according to the diets. Fish fed with the control diet were positioned on the
272 left side of the map, while those with 50% of HI meal were located on the right. The
273 HI25 samples were located closed to the origin of the component. Along the second
274 component (18% of explained variance), sample position reflects the sensory variability
275 due to the biological variability of individuals within the same diet. Considering the
276 distribution of samples in the perceptual space, it appears that the differences among
277 samples due to different diets are more evident than those perceived between different
278 individuals fed the same diet. Thus, the individual evaluations belonging to the same
279 diet were treated as repetition.

280 The mixed ANOVA model on the intensity data of the sensory attributes were
281 performed, in order to estimate the sample effect (three levels: HI0, HI25, HI50) (Table
282 5). A significant sample effect of the diets was found for 12 out of 19 attributes
283 evaluated. No significant effects of assessor \times product interactions were found for the
284 significant attributes (data not reported). Results reported in Table 5 showed that the
285 main differences were found between the control (HI0) and HI50 samples, while HI25
286 expressed some similarities with HI0 for some attributes and with HI50 for the others.
287 Considering the aroma-related attributes, the perceived intensities of boiled fish, algae
288 and overall aroma were significantly higher in HI0 than HI50 samples ($p < 0.001$). On
289 the other hand, the fresh fish aroma showed a significantly higher intensity in HI25 than
290 HI0 and HI50 ($p < 0.05$). Metallic aroma was higher in samples from FM partially
291 replaced with insect proteins diets. Texture attributes resulted significantly more intense
292 in HI50 than HI0 and HI25 ($p < 0.05$). Indeed, samples obtained by fish fed the 50% of
293 insect meal inclusion diet were juicier, more tender and melting more in mouth than HI0
294 samples. Overall aroma intensity tended to significantly decrease with the increasing of
295 insect protein inclusion.

296 Boiled fish flavour and sweet taste were perceived as more intense in HI0
297 samples, with respect to individuals fed insect meal diets ($p < 0.05$). The addition of
298 *Hermetia illucens* prepupae meal also induced a significant increase in overall flavour
299 intensity, independently from HI concentration. Moreover, metallic flavour intensity
300 increased with the increase of HI meal content in the diets.

301

302 Temporal Dominance of Sensations

303 Mean TDS curves of the samples from the three diets are reported in Figures 2–4
304 for HI0, HI25 and HI50 groups, respectively. In general, the curves showed that the
305 texture attributes dominated the first part of evaluation (0 to 15 seconds), followed by

306 flavour and taste attributes. In HI0 samples (Figure 1) tenderness and fibrousnesses
307 clearly dominated the first part of evaluation. On the other hand, only tenderness clearly
308 dominates the dynamic profile of HI25 (Figure 3) and HI50 samples (Figure 4).
309 Furthermore, it appears that the dominance of juiciness is mainly related to FM partial
310 replacement with insect proteins. Flavour of HI0 samples was dominated by boiled fish
311 and algae flavours even at a lower extent, umami taste was the sensations mostly
312 dominating the after taste. In samples HI25, boiled fish clearly dominated the profile
313 together with algae, metallic flavours and umami. The dynamic profile of HI50 appears
314 complex with several descriptors perceived as dominant at the same time (boiled fish,
315 algae, metallic, umami). Even though umami resulted as the most important attribute in
316 the aftertaste of all samples however some differences among diets have been observed.
317 Indeed, while in HI0 the umami was the only dominant attribute, in HI25 the metallic
318 flavour was dominant and in HI50 the boiled fish flavour persisted until the end of
319 evaluation.

320

321 Physical and chemical characterization of fish fed different diets

322 Table 6 reports results of physical and chemical parameter analyses. No
323 significant effect of diets was observed for both cooking loss and WB shear force,
324 indicating that, from an instrumental standpoint, the samples lose the same amount of
325 water during cooking and were equally soft. Proximate composition of cooked samples
326 was not significantly affected by diets of fish ($p>0.05$). On the contrary, the sum of the
327 principal groups of the fatty acids showed differences associated with the experimental
328 diets. It is of note that HI50 fish have the significantly highest level of saturated fatty
329 acids (SFA), followed by HI25. The HI0 had the significantly lowest content of SFA
330 and the significantly highest content of PUFA ω 3, showing an inverse relationship with

331 fish fed *Hermetia illucens* inclusion diets. MUFA and PUFA showed a similar trend,
332 significantly decreasing with the increase of HI meal concentration in diets.

333

334 Relationship between instrumental and sensory analyses

335 The correlation loadings plot in Figure 5 summarizes the main trend of sensory,
336 chemical and physical variables of the samples obtained from fish fed different diets,
337 highlighting the relationship between sensory and instrumental parameters. The
338 explained variance after the first two components (PC) account of 53%. PC1 (37% of
339 explained variance) separated samples without *Hermetia illucens* inclusion in diet from
340 samples fed including HI prepupae meal. PC2 seemed to further separate samples that
341 have different content of HI. The predominant differences between the samples were
342 due to the fatty acids (FAs), mainly SFA that were negatively related to PUFAs. SFA
343 resulted positively correlated to metallic aroma/flavour, overall flavour and tenderness
344 (negative part of PC1), as well as protein and ash content. The positive part of PC1
345 showed the relationship between PUFA, PUFA ω 3, MUFA and boiled fish flavour and
346 overall aroma. PUFA ω 6 seemed highly related to algae flavour, loaded on the positive
347 part of the second component. At the same time, juiciness and melt in mouth attributes
348 were strongly related to PUFA ω 6 and moisture content. WB-shear force did not play a
349 relevant role in this PCA, as expected considering the lacking of significant differences
350 detected with analysis of variance.

351

352 **DISCUSSION**

353 Sensory evaluation

354 Terms freely generated by assessors to describe fish sensory properties are not
355 associated to negative hedonic valence, thus indicating that FM partial replacement with

356 insect meal did not induce the perception of sensory defects or off-flavours. According
357 to sensory results, differences among diets have been observed. DA showed significant
358 differences in terms of aroma, flavour and texture. These results disagree with previous
359 findings reported in literature. For example, no sensory significant differences have
360 been found with inclusion of insect meal in diets on Atlantic salmon.¹⁸ Performing a
361 triangle test on rainbow trout fed diet with different content of insect meal, Sealey et
362 al.¹⁷ did not find any significant differences. In these studies, differences in FA
363 composition were detected, and the lacking of significant differences in sensory
364 proprieties was quite unexpected since differences in FA composition affect the sensory
365 profile.^{20,21} Possibly these results reflect the lack of power of the adopted sensory
366 techniques. The results of the present work further confirm DA as a powerful sensory
367 descriptive technique, providing the accurate description of sample sensory properties.

368 The dynamic analysis of sensory proprieties confirmed the differences between
369 the groups of fish fed different diets. TDS results partially confirmed the results
370 obtained by DA, and allowed a better understanding of the perception of sensory
371 proprieties during all the chewy process. Fibrousnesses intensity was not significantly
372 different amongst trout samples but this sensation appears to be much more important in
373 HI0 than in HI25 and HI50 samples. The inclusion of HI prepupae meal in diets led to
374 the perception of a more complex sensory profile with several flavour sensations
375 dominating the perception at the same time. Dominance of metallic flavour
376 characterized HI25 and HI50 samples in respect to HI0. This sensation can be seen as
377 unfamiliar or as unexpected in fish thus catching the assessor attention despite its
378 moderate intensity value. Dominance values indicated that the importance of a sensation
379 during food consumption is not necessarily the same as that indicated by intensity
380 ratings from static sensory profiles.³⁷ Thus, the use of the TDS method for the sensory

381 characterization of fish samples provides information which complements those from
382 DA studies.

383

384 Physical and chemical characteristics and relationship with sensory profile

385 The partial replacement of FM with HI meal in diets for fish feeding, as
386 alternative source, seems to have effects on qualitative aspects of fillet, in terms of
387 sensory and physico-chemical characteristics.^{16,17,23} In the present study, the
388 instrumental physical differences concerning the parameters investigated were not
389 identified. The *H. illucens* meal is a high-value feed source, rich in protein and fat. The
390 fat amount of black soldier fly larvae is extremely variable and depends on the feeding
391 substrate and development stage of the insect. Further, their FA composition depends on
392 the FA composition of the diet utilised for larvae rearing.³ The lipid content of HI
393 affected the chemical composition of fish fillet, when the FM was partially replaced by
394 the insect meal. In this study, it seems that the inclusion of HI in diets implies a change
395 in FA profile of fish fillet, especially increasing the incidence of SFAs. On the other
396 hand, the PUFA incidences diminish when HI inclusion increases compared to control
397 samples. This trend was also observed in previous studies on Atlantic salmon¹⁸ and
398 rainbow trout,¹⁷ where the amount of whole-body SFAs increased employing diets
399 containing increased amount of HI. Regarding proximate composition, in our trial no
400 significant differences in fillets from different groups of fish were noted. Contrariwise,
401 Sealey et al. observed that fillet moisture and lipid composition were significantly
402 altered by replacement of dietary FM with black soldier fly prepupae meal in rainbow
403 trout.¹⁷ They reported that fish fed diets containing HI had significantly greater moisture
404 and lower lipid in muscle in comparison with fish fed the control diet. Even though
405 these analyses were conducted on raw muscle, while in our study the analysis was
406 performed on cooked fillets, the results of this work are partly in line with these

407 previous findings, since a trend for a lower lipid content in fillet with increasing
408 inclusion of insect meals, even if not significant, was also observed.

409

410 Relationship between sensory and instrumental analyses

411 The compositional differences of the diets, i.e. lipid content, FA profile and
412 proximate composition, can have affected the sensory properties. These variations in
413 diet modify, in particular, lipid content and composition of fish muscle. Overall aroma
414 and flavour intensity are both dependent on final product lipid content.¹² This study has
415 highlighted the relationship between sensory and physico-chemical parameters (Figure
416 5). FAs, flavour and texture attributes showed the main relationships. SFA increase in
417 the fish fillet with the increased inclusion of HI in the diets, and it seems to be
418 correlated to flavour and texture in fish flesh, in agreement with previous study.¹⁵ The
419 rise of fatty acids had an effect on tenderness of fish meat as confirmed also by
420 Grigorakis et al.¹¹ and Rincón et al.¹² findings. Valente et al.³⁸ found a significant
421 relationship between lipid content and both fatty flavour and perception of fatty texture.
422 Additionally, lipid content and FA profile of fillets have a connection to flesh texture³⁹
423 and they affect texture attributes, mainly juiciness and tenderness.¹¹ However, this
424 relationship with tenderness measured with Warner-Bratzler shear force was not
425 revealed in the case of this trial samples. Water contents contributed to juiciness and
426 melting in mouth of the fish samples, in agreement with a previous work findings.¹²
427 Concerning the flavour modification, Grigorakis et al.¹¹ showed that fat content strongly
428 affects the mouth impression and volatile compounds that were also correlated to
429 differences in sensory taste.

430

431 **CONCLUSION**

432 Sensory description of fish samples indicated that HI inclusion induces
433 significant differences in the perceived profile. Furthermore, HI inclusion in the diet did
434 not induce the perception of sensations relevant to defects or off-flavours. The effect of
435 diets was highlighted both by DA and TDS descriptions and the information obtained
436 appear complementary. The strict relationship between sensory profile and fatty acid
437 composition was also confirmed by the results obtained. Further study will be necessary
438 to understand if the highlighted differences in sensory properties of fish fed diets
439 characterized by different protein sources would be reflected in liking judgements by
440 consumers.

441

442 **ACKNOWLEDGMENTS**

443 The research was funded by University of Florence, Ateneo Research Project
444 (2014) and by University of Turin, ex-60% grant (2014).

445

446 **REFERENCES**

- 447 1 FAO, *The State of World Fisheries and Aquaculture. Opportunities and*
448 *Challenges*, FAO, Rome, pp. 3. (2014).
- 449 2 Tacon AGJ, Hasan MR and Metian M, Demand and supply of feed ingredients
450 for farmed fish and crustaceans: trends and prospects, in *FAO Fisheries and*
451 *Aquaculture Technical Paper* No. 564, Rome pp. 87 (2011).
- 452 3 Makkar HP, Tran G, Heuzé V and Ankers P, State-of-the-art on use of insects as
453 animal feed. *Anim Feed Sci Tech* **197**:1-33 (2014).
- 454 4 Veldkamp T. & Bosch G., Insects: a protein-rich feed ingredient in pig and
455 poultry diets. *Anim Fron* **5**:45-50 (2015).

- 456 5 Bovera F, Loponte R, Marono S, Piccolo G, Parisi G, Iaconisi V, Gasco L and
457 Nizza A, Use of *Tenebrio molitor* larvae meal as protein source in broiler diet:
458 effect on growth performance, nutrient digestibility, carcass and meat traits. *J*
459 *Anim Sci* **94**:639-647 (2016).
- 460 6 van Huis A, Van Itterbeeck J, Klunder H, Mertens E, Halloran A, Muir G and
461 Vantomme P, Edible insects – Future prospects for food and feed security. *FAO*
462 *Forestry Paper* No. 171, Rome pp. 201 (2013).
- 463 7 van Huis A, Potential of insects as food and feed in assuring food security. *Annu*
464 *Rev Entomol* **58**:563-583(2013).
- 465 8 Henry M, Gasco L, Piccolo G and Fountoulaki E, Review on the use of insects
466 in the diet of farmed fish: Past and future. *Anim Feed Sci Tech* **203**:1-22 (2015).
- 467 9 Roncarati A, Gasco L, Parisi G and Terova G, Growth performance of common
468 catfish (*Ameiurus melas* Raf.) fingerlings fed *Tenebrio molitor* meal diet.
469 *Journal of Insects as Food and Feed* **1**:233-240 (2015).
- 470 10 Gasco L, Henry M, Piccolo G, Marono S, Gai F, Renna M, Lussiana C,
471 Antonopoulou E, Mola P, Chatzifotis S, *Tenebrio molitor* meal in diets for
472 European sea bass (*Dicentrarchus labrax* L.) juveniles: growth performance,
473 whole body composition and in vivo apparent digestibility. *Anim Feed Sci*
474 *Technol* **220**:34-45 (2016).
- 475 11 Grigorakis K, Taylor KDA and Alexis MN, Organoleptic and volatile aroma
476 compounds comparison of wild and cultured gilthead sea bream (*Sparus*
477 *aurata*): sensory differences and possible chemical basis. *Aquaculture* **225**:109-
478 119 (2003).
- 479 12 Rincón L, Castro PL, Álvarez B, Hernández M, Álvarez A, Claret A, Guerrero L
480 and Ginés R, Differences in proximal and fatty acid profiles, sensory

- 481 characteristics, texture, colour and muscle cellularity between wild and farmed
482 blackspot seabream (*Pagellus bogaraveo*). *Aquaculture* **451**:195-204 (2016).
- 483 13 Olsson GB, Olsen RL, Carlehög M and Ofstad R, Seasonal variations in
484 chemical and sensory characteristics of farmed and wild Atlantic halibut
485 (*Hippoglossus hippoglossus*). *Aquaculture* **217**:191-205 (2003).
- 486 14 Sveinsdóttir K, Martinsdóttir E, Green-Petersen D, Hyldig G, Schelvis R and
487 Delahunty C, Sensory characteristics of different cod products related to
488 consumer preferences and attitudes. *Food Qual Prefer* **20**:120-132 (2009).
- 489 15 Turchini GM, Mentasti T, Caprino F, Panseri S, Moretti VM and Valfré F,
490 Effects of dietary lipid sources on flavour volatile compounds of brown trout
491 (*Salmo trutta* L.) fillet. *J Appl Ichthyol* **20**:71-75 (2004).
- 492 16 Turchini GM, Torstensen BE and Ng W-K, Fish oil replacement in finfish
493 nutrition. *Rev Aquacult* **1**:10-57 (2009).
- 494 17 Sealey WM, Gaylord TG, Barrows FT, Tomberlin JK, McGuire MA, Ross C
495 and St-Hilaire S, Sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fed
496 enriched black soldier fly prepupae, *Hermetia illucens*. *J World Aquacult Soc*
497 **42**:34-45 (2011).
- 498 18 Lock ER, Arsiwalla T and Waagbø R, Insect larvae meal as an alternative source
499 of nutrients in the diet of Atlantic salmon (*Salmo salar*) postsmolt. *Aquacult*
500 *Nutr.* doi:10.1111/anu.12343 (2015).
- 501 19 Grigorakis K, Compositional and organoleptic quality of farmed and wild
502 gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) and
503 factors affecting it: a review. *Aquaculture* **272**:55-75 (2007).
- 504 20 Izquierdo MS, Montero D, Robaina L, Caballero MJ, Rosenlund G and Ginés R,
505 Alterations in fillet fatty acid profile and flesh quality in gilthead sea bream

506 (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid
507 profiles by fish oil feeding. *Aquaculture* **250**:431-444 (2005).

508 21 Montero D, Robaina L, Caballero MJ, Ginés R and Izquierdo MS, Growth, feed
509 utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed
510 diets containing vegetable oils: a time-course study on the effect of a re-feeding
511 period with 100% fish oil diet. *Aquaculture* **248**:121-134 (2005).

512 22 de Francesco M, Parisi G, Pérez-Sánchez J, Gómez-Réqueni P, Médale F,
513 Kaushik SJ, Mecatti M and Poli BM, Effect of high level fish meal replacement
514 by plant proteins in gilthead sea bream (*Sparus aurata*) on growth and
515 body/fillets quality traits. *Aquacult Nutr* **13**:361-372 (2007).

516 23 St-Hilaire S, Sheppard C, Tomberlin JK, Irving S, Newton L, McGuire MA,
517 Mosley EE, Hardy RW and Sealey W, Fly prepupae as a feedstuff for rainbow
518 trout, *Oncorhynchus mykiss*. *J World Aquacult Soc* **38**:59-67 (2007).

519 24 Verbeke W, Functional foods: Consumer willingness to compromise on taste for
520 health? *Food Qual Prefer* **17**:126–131 (2006).

521 25 Amberg S and Hall T, Communicating risks and benefits of aquaculture: a
522 content analysis of U.S. newsprint representations of farmed salmon. *J World*
523 *Aquacult Soc* **39**:143-157 (2008).

524 26 Dinnella C, Masi C, Naes T and Monteleone E, A new approach in TDS data
525 analysis: A case study on sweetened coffee. *Food Qual Prefer* **30**:33-46 (2013).

526 27 Lawless HT and Heymann H, *Sensory evaluation of foods: Principles and*
527 *practices*. In Chapman & Hall (Eds.), New York, USA: Springer (1998).

528 28 Pineau N, Schlich P, Issanchou S, Imbert A, Cordelle S, Mathonnière C,
529 Rogeaux M, Etiévantc P and Kösterf E, Temporal dominance of sensations:
530 Construction of the TDS curves and comparison with time-intensity. *Food Qual*
531 *Prefer* **20**:450-455 (2009).

532 29 Piggott JR, Dynamism in flavour science and sensory methodology. *Food Res*
533 *Int* **33**:191-197 (2000).

534 30 Piggott JR and Watson MP, A comparison of free-choice profiling and the
535 repertory grid method in the flavor profiling of cider. *J Sens Stud* **7**:133-145
536 (1992).

537 31 Veland JO and Torrissen OJ, The texture of Atlantic salmon (*Salmo salar*)
538 muscle as measured instrumentally using TPA and Warner-Bratzler shear test. *J*
539 *Sci Food Agr* **79**:1737-1746 (1999).

540 32 AOAC, *Official Methods of Analysis of AOAC International*, 17th Association of
541 Official Analytical Chemists, Gaithersburg. Maryland, USA (2000).

542 33 Folch J, Lees M and Sloane-Stanley GH, A simple method for the isolation and
543 purification of total lipids from animal tissues. *J Biol Chem* **226**:497-509 (1957).

544 34 Næs T, Brockhoff P and Tomic O, *Statistics for sensory and consumer science*.
545 John Wiley & Sons (2011).

546 35 Martens H and Martens M, *Multivariate Analysis of Quality: an Introduction*.
547 New York: John Wiley & Sons Ltd Ed (2001).

548 36 Rosner B, *Fundamentals of Biostatistics*, 4th ed. by Belmont: Duxbury Press
549 (1995).

550 37 Dinnella C, Masi C, Zoboli G and Monteleone E, Sensory functionality of extra-
551 virgin olive oil in vegetable foods assessed by Temporal Dominance of
552 Sensations and Descriptive Analysis. *Food Qual Prefer* **26**:141-150 (2012).

553 38 Valente LMP, Cornet J, Donnay-Moreno C, Gouygou JP, Bergé JP, Bacelar M
554 and Cardinal M, Quality differences of gilthead sea bream from distinct
555 production systems in southern Europe: intensive, integrated, semi-intensive or
556 extensive systems. *Food Control* **2**:708-717 (2011).

557 39 Johansson L, Kiessling A, Kiessling KH and Berglund L, Effects of altered
558 ration levels on sensory characteristics, lipid content and fatty acid composition
559 of rainbow trout (*Oncorhynchus mykiss*). *Food Qual Prefer* **11**:247-254 (2000).