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Maïa Meurillon, Chloé Anderson, Magaly Angénieux, Frédéric Mercier, Nathalie Kondjoyan, Erwan Engel

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2 **Sensory acceptability of antioxidant-**
3 **based formulations dedicated to mitigate**
4 **heterocyclic aromatic amines in cooked**
5 **meat**

6

7

8 **MEURILLON, Maia**^{1*}; ANDERSON, Chloé¹; ANGENIEUX, Magaly¹; MERCIER,
9 Frédéric¹; KONDOYAN, Nathalie¹; ENGEL, Erwan¹

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11

12 ¹ *INRAE, UR370 QuaPA, Microcontaminants, Aroma and Separation Science Lab, 63122*

13

Saint-Genès-Champanelle, France

14

15

** Corresponding author: maia.meurillon@inrae.fr*

16

☎: +33 (0)4 73 62 41 65

17 **Abstract:**

18 In a previous work, we studied the inhibition of heterocyclic aromatic amine formation by
19 natural ingredients rich in antioxidants: caper, oregano, wine and green tea. This present work
20 aimed to assess the sensory impact of the addition of these ingredients in ground beef patties.
21 The best liked formulations were determined by a hedonic scoring. Caper and oregano were not
22 significantly different from the standard, the most appreciated, due to their congruency with
23 cooked meat. Direct dissimilarity assessment was performed to evaluate the overall, gustative
24 and odor differences between formulations. Olfactive differences were evidenced as key drivers
25 of these differences. Standard and caper were close while oregano was found significantly
26 different from them. A fast sensory profiling permitted to evidence that aromatic plant note was
27 discriminant for oregano formulation. Gas chromatography-mass spectrometry/multiboost
28 olfactometry identified the distinctive odor-active compounds of the formulations as pyrazines
29 and sulfide for oregano formulation and ester for caper formulation.

30

31 **Keywords:** Heterocyclic aromatic amines, sensory analysis, hedonic scoring, direct
32 dissimilarity assessment, sensory profiling, gas chromatography-mass spectrometry/multiboost
33 olfactometry.

34

35 **1. Introduction**

36 The preparation and cooking of meat products have an important effect on their microbial load,
37 palatability and composition. However some of those compositional changes such as the
38 formation of heterocyclic aromatic amines (HAAs) have been linked to the promotion of certain
39 cancers in meat consumers. Consequently in 2015, the International Agency for Research on
40 Cancer classified the consumption of red meat and processed meat as probably carcinogenic
41 (group 2A) and carcinogenic (group 1) to humans, respectively (Bouvard, et al., 2015). To

42 address this issue, mitigation strategies to reduce HAA impact on human health were proposed.
43 One of the most promising approach is the addition of natural antioxidants that limit the
44 formation of HAAs (Meurillon & Engel, 2016). However, to date there is no method for making
45 a reasoned choice of antioxidants. Therefore, in a previous work (Meurillon, et al. 2020), a
46 unique and original method based on medicinal chemistry approaches was developed to select
47 antioxidants best suited to inhibit HAA formation in meat. This method allowed a
48 rationalization of the choice of antioxidants to optimize meat chemical safety but it would only
49 be admissible if these antioxidants are sensorially validated. Indeed, the addition of antioxidants
50 should in no way modify the sensory acceptance of the product or be subject to its rejection by
51 consumers. Previous works studying the sensorial effect of the addition of antioxidants in meat
52 often used descriptive analysis (Melo, et al., 2008; Quelhas, et al., 2010; Vidal, et al., 2020;
53 Viegas, et al., 2012). However, for such method, the need to recruit a trained panel and to further
54 train them on the tested formulations is essential but long and expensive. Even when trained,
55 the number of descriptors and the specific relation to a descriptor can be broad between
56 individuals which brings high variability in the results.

57 The objective of the present paper was to evaluate the sensory impact of the addition of the 4
58 natural ingredients rich in phenolic antioxidants evidenced by our previous work (Meurillon, et
59 al. 2020): caper, oregano, red wine and green tea, in order to further validate their use in
60 mitigation strategies for meat products. For that it was necessary to determine what drove
61 consumer's acceptance and to identify the sensory determinants that were at the origin of the
62 latter. First, a hedonic study was done to determine which formulations among the 4 tested were
63 the most appreciated by consumers. To explain these results, different non-verbal analyses were
64 done to assess overall dissimilarities between the studied formulations and evidence the drivers
65 of these differences. To characterize these differences with descriptive information a fast
66 version of traditional quantitative descriptive analysis (QDA®) was implemented. Finally, the

67 distinctive odor-active compounds and their contribution to the overall odor of the different
68 formulations were determined by DH-GC-MS/80 in order to identify potential determinants of
69 consumer's sensorial acceptance of the chosen formulations.

70

71 **2. Materials and Methods**

72 ***2.1. Sample Preparation***

73 Ground beef was obtained from Société Convivial (Gannat, France). The meat was certified
74 from Charolais cattle and contained 11% fat. Stored at -80°C, it was thawed overnight in the
75 fridge at 4°C the day before preparation.

76 As the aim of this work was to evaluate the sensory impact of the addition of the 4 natural
77 ingredients rich in phenolic antioxidants evidenced by Meurillon, et al. (2020), the same
78 formulations were chosen. The four natural products rich in phenolic antioxidants were
79 purchased from the local supermarket and were comprised of: 'Extra Fine Capers' from Maille,
80 Pinot Noir Burgundy wine bottles (red wine), organic green tea in bags from Naturela and dried
81 oregano from Ducros. They were added in two ways: marinated or blend in. Marinade durations
82 were chosen according to literature data on marinated meat (Busquets, Puignou, Galceran, &
83 Skog, 2006; Melo, et al., 2008) to induce a decrease in HAAs while being realistic with common
84 household practices. Concentrations in natural ingredients were chosen to be realistic from a
85 consumer point of view: high enough to display a potential inhibitory effect based on literature
86 data (Gibis & Weiss, 2012; Friedman, Zhu, Feinstein, & Ravishankar, 2009) but low enough to
87 be accepted in meat recipes.

88 It should be noted that our previous work dealing with the inhibition of process-induced
89 toxicants (HAAs) had tested 3 modalities per formulation (marinade either 2, 4 or 6h or blended
90 with 0.1%, 0.25% or 0.5% of natural product, 3 replications for each formulation and each
91 concentration or marination time) (Meurillon, et al. 2020). In the present work, only the 2 higher

92 modalities were tested as the more concentrated the natural product will be the more it will
93 impact on sensory properties of the meat recipes. And if the sensory properties of the more
94 modified meat products are accepted by consumers, it can be assumed that products less
95 modified and closer to standard ground beef patties will be all the more accepted. For red wine
96 and green tea samples, batches of 4 ground beef patties of 125g each were fully marinated
97 together in either solution for 4 or 6 hours, then mixed four times for 20 seconds. For the caper
98 and oregano samples, the necessary amount of natural product (0.25% and 0.5% mass
99 concentration) was first manually grounded in a stainless-steel mortar and then added to meat
100 patties (batches of 4x125g) and mixed four times for 20 seconds.

101 After mixing, patties of 115g of the same dimension were shaped with a round stainless steel
102 ring (12 cm diameter). The patties were then put at -80°C for 45min, vacuumed sealed and
103 stored at -18°C in the freezer.

104 For the samples intended for DH-GC-MS/80 analysis, beef patties were prepared as above but
105 were given the highest concentration of natural products (6h marination and 0.5% dry weight)
106 and stored at -80°C. There were six samples including four formulations rich in antioxidants,
107 one standard and one water-marinated standard. The day before the analyses, the samples were
108 thawed at 4°C overnight. The next morning, they were cooked under the conditions described
109 in part 2.2. 10 g of cooked sample were taken, 5g from the center of the steak and 5g from the
110 edge with some juice to be representative of the whole sample.

111

112 ***2.2. Sample cooking***

113 Sample preparation was done under common cooking method in order to best mirror household
114 practices for meat preparation. Patties were removed from the freezer (-18°C) and stored
115 overnight in the fridge at 4°C the day before manipulation in order to defrost gently. They were
116 then cooked in a stainless steel frying pan (20 cm diameter, 2 patties of a same modality in a

117 given frying pan) on a controlled-temperature induction hob (Bosch Electroménager, Saint-
118 Ouen, France). Cooking conditions were based on the work of Planche et al. (2017) to simulate
119 medium meat, which corresponded to a core temperature of 70°C according to the WHO
120 recommendations for ground meats. These cooking conditions corresponded to 14 min heating
121 (turned over three times) at 200°C at the bottom of the pan (Planche, et al., 2017). Temperatures
122 at the core of the meat and at the bottom of the pan were monitored by thermocouples (RS
123 Components, Beauvais, France). This method of cooking (Planche, et al., 2017) was developed
124 with the aim of being realistic, standardized and reproducible. In order to ensure the
125 reproducibility between the different cooking batches, the core temperature was controlled
126 systematically throughout the experiments by thermocouples and was found stable. Therefore,
127 we considered the cooking as homogeneous in a given batch and from one batch to another.
128 The patties were then cut in 4 or 6 pieces and placed on glass Petri dish for testing.

129

130 *2.3. Sensory analysis*

131 All the evaluations were conducted in air-controlled rooms. Sessions of 35 min were spread
132 over a period of 4 weeks. The participants for hedonic scoring were recruited from the interns
133 and staff. Participants were aged 18 to 65 years old. They were all meat consumers but had no
134 experience in hedonic scoring, assessing direct dissimilarity measures and sensory profiling.
135 Among these 59 participants, 15 persons were selected to conduct non-verbal analysis and fast
136 sensory profiling based on their ability to recognize odors and classify their intensity (see 2.3.2).
137 In order to make a quick characterization of our different formulations, no repetition was carried
138 out by the judges during sensory analysis.

139 *2.3.1. Hedonic Scoring*

140 Hedonic scoring was used to assess preferences of the global population for the chosen
141 formulations. In our experiments, 59 meat consumers were recruited to participate in a series

142 of two sessions over a period of two weeks. According Bonaïti, et al. (2005), each judge
143 evaluated a given product only once. Each session was composed of formulations of two
144 different natural products rich in antioxidants at two concentrations and a standard. For
145 logistical reasons (room size for the evaluations, cooking management to guarantee
146 homogeneous conditions, etc.), each session (6 in total) regrouped 10 or 9 participants and had
147 5 tasting rounds (1 standard and 2 formulations at 2 different concentrations). During a given
148 session, samples of the same modality were issued from the same cooking batch. A week later,
149 the same panelists came for a second session (again 6 sessions of 10 or 9 participants) during
150 which they tested the 2 other formulations at 2 different concentrations and the standard. The
151 samples were coded with a 3-digit number and given in a monadic way (according to a Williams
152 Latin Square design (MacFie, et al., 1989)) to be rated on a 9 point hedonic scale (1, dislike
153 extremely; 5, neither like nor dislike; 9, extremely pleasant). The judges were asked to rinse
154 their mouth with the help of crackers and water. The same consumers were asked to come back
155 the following week to try two other formulations. Thus, at the end of the 2 sessions, each
156 panelist tasted all the formulations at the different concentrations/marination times as well as
157 the standard.

158 2.3.2. *Direct dissimilarity assessment*

159 The non-verbal procedures used in this research work were directly inspired by Bonaïti *et al.*
160 (2005) and their method of direct dissimilarity assessment in order to evaluate the overall,
161 gustative and odor differences between the different formulations. To assess the best panelists
162 among the 59 participants of the hedonic scoring, samples of diluted natural products in water
163 at three concentrations (1%, 0.5%, and 0.25%) were placed in brown containers to be evaluated
164 for odor. The 59 panelists were instructed to smell the containers as many times as they wanted
165 and were asked to provide a descriptor or the nature of the product and to rank the solutions
166 according to their intensity. The 15 panelists who provided close descriptors and best ranking

167 were selected for the next step but the results obtained were not further analyzed. The 15
168 selected panelists were instructed to estimate the overall dissimilarity between samples by
169 positioning them in a square paper map of 60 x 60cm. This part was divided into two sessions
170 in the same week. The first session only assessed overall dissimilarity while the second assessed
171 olfactive and gustative dissimilarity. For the later one, consumers were asked to put a nose clip
172 to assess the relative contribution of smell in the results of dissimilarity while in the olfactive
173 step, they were not permitted to taste the formulations. Samples were presented in the center of
174 the paper and panelists were allowed to smell and/or taste the samples as many times as they
175 wanted without respecting any specific order. In this non-verbal analysis, panelists were asked
176 to place the formulations on a white sheet of paper based on their dissimilarities in terms of
177 flavor and odor. Coordinates were calculated for each sample and each assessor. The largest
178 between-sample distance was used to normalize distances that were obtained from each
179 panelist. Normalized distances were then compiled to obtain an average distance matrix.

180 *2.3.3. Fast sensory profiling*

181 In order to supplement the information of the non-verbal analysis with descriptive information
182 without resorting to a too cumbersome method, we implemented a lighter sensory profiling
183 analysis inspired from a fast version of traditional QDA®. The focus of this section was to
184 formulate sensory descriptors and link them to the results found for non-verbal analysis.
185 Therefore, if it followed the quantitative descriptive rationale, it did not strictly follow the strict
186 conditions required. Thus, standardized QDA® sequences require panelists to be trained and
187 follow other product-related training sessions (Murray, et al., 2001). To stay consistent with the
188 “fast” logic of non-verbal analyzes, a full quantitative descriptive analysis could not be
189 conducted. Indeed, to do so, 10-12 trained panelists should have been trained intensively before
190 establishing the lexicon and quantifying the descriptors. In this fast sensory profiling though,

191 panelists were trained only once to develop a consensual vocabulary and had only little previous
192 experience in sensory analysis.

193 Judges from the previous step (n=15) with stronger sensory capacities were asked to come for
194 two more sessions. First the panelists were trained during an orientation session and they came
195 back two days later to evaluate the samples based on the training session instructions. The aim
196 of the first session was to develop descriptive terms that could describe each sample and agree
197 on the definition of each term. In the training session, samples with the highest concentration
198 of antioxidant were used to facilitate the generation of terms. The session was monitored in
199 order to agree on a consensual vocabulary. The final list was composed of 16 terms describing
200 taste (4), texture (2) and aroma (10). Some of them were coherent with cooked meat aromas but
201 others seemed to originate from the addition of natural ingredients. Sour, salty, bitter and umami
202 were the four gustatory attributes recorded. Typical aroma and odor descriptors from cooked
203 meat were described with grilled, fat-like, mushroom, caramel, bloody, rancid and meaty and
204 matched the data displayed by Adhikari et al. in the lexicon for beef flavor (Adhikari, et al.,
205 2011). Other attributes were also included as “aromatic plants”, “winy” and “fruity”. In
206 addition, the panelists included two physical attributes: “juiciness” and “cohesiveness”. In the
207 second session, semi-trained panelists received the concentrations chosen in the hedonic step,
208 including two standards (unmarinated and water marinated) and were instructed to rate the
209 intensity of each term using a 10 cm unstructured linear scale from ‘no perception’ to ‘strong
210 perception’. For this session, the samples were presented in a monadic way to balance report
211 and position effects. Between samples, they were instructed to eat some crackers and rinse their
212 mouth with water.

213

214 ***2.4. Dynamic headspace-gas chromatography-mass spectrometry/multiboath***
215 ***olfactometry (DH-GC-MS/80)***

216 The odor-active compound determination was carried out according to the work of Giri *et al.*
217 (2015) using a unique GC-MS/80 system accumulating the performance of 8 judges
218 simultaneously on the same sample to detect what were the most significant odor zones in the
219 chromatogram.

220 The volatile compounds were extracted by dynamic headspace (Tekmar, Cincinnati, OH, USA)
221 coupled to gas chromatography (GC 6890A, Agilent Technologies) hyphenated to a quadrupole
222 mass detector (HP4440 system, Agilent Technologies) according to Giri *et al.* (2015). The
223 sample was purged with helium (purity 99.995%) at 30°C for 1h with a flow rate of 40 mL.min⁻¹
224 using a Tenax trap. The volatile components were then desorbed from the trap at 180°C for 10
225 min using helium (purity 99.9995%) and sent into a cryofocalization area cooled to -150°C with
226 liquid nitrogen. After injection by heating at 180°C for 2 min, the volatile components were
227 separated on a non-polar GC column (60 m × 0.32 mm × 1 µm d_f; RTX-5MS; Restek,
228 Bellefonte, PA, USA) and smelled according to Berdagué *et al.* (2007). The sniffer selection
229 and olfactory session were conducted according to Giri *et al.* (2015). The olfactometry analyses
230 lasted 35 min and the scan speed of acquisition was 1 scan.s⁻¹.

231

232 **2.5. Data Analysis**

233 Data were analyzed using Statistica software 10.0 (Statsoft, Maisons-Alfort, France) and
234 AcquiSniff[®] software (INRA, Clermont-Ferrand, France).

235 **2.5.1. Hedonic Scoring**

236 A categorical one-way ANOVA (formulation) was performed on hedonic mean scores
237 according to the model: attribute = formulation. The judges weren't considered as explanatory
238 factor and therefore included as a random term. When significant differences were found ($P <$
239 0.05), hedonic mean scores were compared using Duncan's multiple comparison test.

240 **2.5.2. Direct dissimilarity assessment**

241 Direct dissimilarity assessment data were analyzed through multidimensional scaling (MDS)
242 as proposed by Bonaïti *et al.* (2005). MDS raw data were the coordinates of the points for each
243 formulation that was expressed into a unique matrix of distances for the whole panel. This
244 method provided a barycenter of the accumulated results. As a result of a MDS analysis, the
245 formulations were represented by a two-dimensional map configuration. The distance between
246 the barycenters of each group in the spatial arrangement translated the relative dissimilarity
247 between formulations. MDS reduced the complexity of the data set and permitted a visual
248 appreciation of the underlying relational structures contained therein (Hout, *et al.*, 2013).

249 *2.5.3. Fast sensory profiling*

250 In order to make fast sensory profiling of our different formulations, no repetition was carried
251 out by the judges. Therefore, no interactions could be measured in addition to the effects of
252 judges and products (*i.e.* formulations). Based on the assumption that the core temperature and
253 the cooking time were controlled and given that the same cooking equipment was used, the
254 cooking conditions were considered homogeneous between the batches. Two-way ANOVA
255 (formulation, judge) was performed on mean attribute intensities according to the model:
256 attribute = formulation + judge. When significant differences were found ($P < 0.05$), mean
257 attribute intensities were compared using Duncan's multiple comparison test.

258 *2.5.4. Dynamic headspace-gas chromatography-mass spectrometry/multiboath*

259 *olfactometry (DH-GC-MS/80)*

260 Olfactometric data were acquired and processed with AcquiSniff[®] software (Tournayre &
261 Berdagué, 2003). After running the DH-GC-MS/80 (Berdagué, *et al.*, 2007) on the six samples,
262 over 600 terms were generated by the 8 panelists. Panelists were free to choose any terms but
263 given the congruence of some descriptors and for a better representation of the results on the
264 aromagram, the vocabulary was classified into 12 odor poles (table 1). By its coupling with a
265 mass spectrometer, the DH-GC-MS/80 enabled to identify the odor-active compounds and to

266 assess their contribution to the overall odor/aroma of the product. The matching of the resulting
267 candidate compounds and the odor descriptors generated by the trained panelists was assessed
268 thanks to a library of aroma descriptors and retention time found at thegoodscentcompany.com
269 and the nature of the peaks was proposed.

270 A one-way ANOVA was carried on all the intensity ratings from the eight judges for each odor-
271 active compound to find the discriminatory zones according to the model: attribute =
272 formulation. Those zones were presented on an ANOVAgam and the 20 first most
273 discriminative zones were selected according to Fischer-F value. From the 20 most significantly
274 discriminative peaks, only 10 were highly discriminant ($P < 0.001$ according to ANOVA).

275

276 **3. Results and Discussion**

277 *3.1. Acceptability of the different formulations: Hedonic scoring*

278 The first part of the study determined the preferred formulations that were then used in the
279 subsequent sensory work. The four formulations (caper, oregano, green tea and wine) at two
280 different concentrations were tested by the panelists along with a standard. The results of the
281 hedonic rating are presented in figure 1.

282 The results showed a great disparity of the hedonic scores between the different formulations.
283 The standard appeared to be the most preferred among the samples. A preference for standard
284 preparation is frequently reported in hedonic experiments of new formulations and is mostly
285 due to a reluctance to ingest novel food of unknown safety. This food neophobia mechanism
286 leads consumers to down rate unfamiliar food compared to well-known consumed products
287 (Marcontell, et al., 2003; Raudenbush & Frank, 1999; Tuorila & Hartmann, 2020).

288 Interestingly, two of our formulations, caper 0.5% and oregano 0.25 %, were non-significantly
289 different from the standard. This finding can be explained by the congruence of capers and
290 oregano with ground beef patties. These ingredients could be more commonly used for ground

291 beef preparations and therefore more accepted by consumers. Caper 0.25% and oregano 0.5%
292 were non-significantly different from the two other concentrations (caper 0.5% and oregano
293 0.25 %).

294 Oregano and caper formulations were preferred over wine and green tea preparations that were
295 significantly different from the standards. From figure 1, wine and green tea did confer a change
296 that was noticeable by consumers as they rated those formulations as the least preferred. Our
297 findings were in accordance with the results of Melo et al. (2008) showing that longer
298 marination time in wine (4 to 6h) would generate unpleasant wine aroma, strong red color and
299 poor overall quality, contrary to 2h marination which resulted in no to little notable differences
300 in sensorial perceptions (Melo, et al., 2008; Quelhas, et al., 2010). Moreover, the low hedonic
301 scores of wine and tea formulations can again be related to the food neophobia phenomenon as
302 those products are not usually used for ground beef preparations in our occidental countries.

303 Given the results of hedonic scoring, the formulations chosen for the sensory analyses
304 undertaken to explain the observed differences of acceptability were the less concentrated in
305 order to be as close as possible of household practices.

306

307 ***3.2. Sensory dimensions responsible for the differences of acceptability***

308 *3.2.1. Non-verbal dissimilarity rating*

309 Figure 2 shows the two dimensions of the 2D-MDS analysis of non-verbal dissimilarity rating
310 data.

311 This non-verbal method presents many benefits compared to more traditional descriptive
312 analyses as it enables the panelists to rate the overall perceptual dissimilarity of the samples
313 without having to choose or rate descriptive terms (Schiffman et al., 1981) and avoids the
314 problem of individual semantic interpretation of attributes and their definitions (Matuszewska
315 et al., 1992).

316 A MDS mapping of flavor dissimilarities was undertaken (figure 2a) to determine if flavor
317 played an important role in the differentiation of the formulations and standards. A similar non-
318 verbal analysis focused on the smell was also produced (figure 2b) to further develop on the
319 odorous compounds responsible for the differentiation of the products and understand odor
320 contribution on formulation discrimination.

321 The MDS map of the flavor dissimilarity profile (figure 2a) showed that wine and oregano
322 formulations were different between themselves (dimension 1) and to the other formulations
323 and standards (dimension 2). Caper formulation, marinated standard and standard were closely
324 related in the second dimension and tea preparation, although close to this second group, was
325 slightly different in the second dimension. Similar results were found for odor dissimilarity
326 profile (figure 2b).

327 The close proximity between caper formulation and standard in both MDS mapping could
328 explain their similar results in hedonic scoring. Non-verbal results highlighted the dissimilarity
329 between oregano formulation and standard but as oregano is congruent with meat preparation,
330 these sensorial differences didn't result in a decrease of consumer's acceptance. On the
331 contrary, as wine and to a lesser extend tea are not congruent with meat preparation, the sensory
332 differences highlighted in the non-verbal results could explain their low acceptability (as shown
333 by the hedonic scoring).

334 It seems that the other two chemical senses contributing to flavor (gustatory and trigeminal) do
335 not provide additional distinctive information with respect to olfaction. This suggests that they
336 also play a more modest role in the hedonic distinction highlighted in the previous paragraph.
337 As a result, this research will focus on the olfactive dimension to find the molecular
338 determinants responsible for the difference of acceptability.

339 *3.2.2. Fast sensory profiling*

340 The objective of this sensory profiling was to formulate sensory descriptors permitting to
341 determine the sensory attributes that may explain the differences pointed out with non-verbal
342 analyses. Table 2 and figure 3 show the results obtained by this original and fast method.

343 The results from the sensory profiling (table 2 and figure 3) displayed some differences among
344 formulations. 8 attributes among the 16 descriptors permitted to discriminate the formulations.
345 Oregano formulation was differentiated from the other formulations and standards by the
346 aromatic plant note as for this attribute, the formulation effect was significant and more than
347 the judge effect according to the Fischer-F values of the 2-way ANOVA.

348 Wine formulation was found significantly less grilled than the standards, caper and oregano
349 formulations. Wine formulation was also found as juicy as the standard but more than standard
350 marinated and green tea formulation. Interestingly, when looking at the winy attribute, wine
351 formulation was differentiated from green tea formulation which was also distinguished from
352 the standards, caper and oregano formulations. Consumer acceptance seems to be closely
353 related to this winy attribute since the more it increases, the more the hedonic score decreases
354 in relation with the lack of congruence of winy note and meat preparations. Wine formulation
355 was also found significantly sourer and fruitier than the others but according to the Fischer-F
356 values of the 2-way ANOVA, the judge effect was of the same order of magnitude as the
357 formulation effect which prevented to conclude on these attributes. In the same way, wine
358 formulation was found less meaty than the others formulations and standards. However, if a
359 significant formulation effect was found that could reflect a measure of congruence, this latter
360 was of the same order of magnitude as the judge effect, so it prevented from a conclusion.
361 However, it is known that the addition of some natural products rich in antioxidants could affect
362 the general profile of meat by impacting on fat oxidation process and consequently reducing
363 some meat flavor notes such as beef aroma or flavor (Delaquis, et al., 1999).

364 This fast sensory profiling inspired from QDA[®] permitted to confirm what was observed in the
365 non-verbal analyses and to refine the results as it permitted to identify qualitative differences
366 that could be used afterwards through GC-O analysis.

367

368 ***3.3. Odor-active drivers of the sensorial acceptability between formulations***

369 *3.3.1. Qualitative comparison of the aromagrams of the different formulations*

370 Figure 4 and supplementary data 1 present the qualitative comparison of the aromagrams of the
371 different formulations with the objective to identify the odor-active compounds responsible for
372 the differences observed in the fast sensory profiling above.

373 The aromagrams with the most differences between them are those of the standard, wine and
374 oregano formulations, which is consistent with the MDS and fast sensory profiling conclusions
375 (figures 2 & 3 and table 2). The “green/aromatic plant” note at 950s present in the aromagram
376 of the standard was absent in the 4 formulations which displayed instead fruity/floral notes at
377 this given retention time. These “green/aromatic plant” notes are often attributed to aldehydes
378 which are generally considered as oxidation markers. The fact that this note is less pronounced
379 in the mitigating formulations may be related to their antioxidant capacities. The wine
380 formulation was distinguished by a strong contribution of fruity/floral notes which could be
381 related to esters which are frequently reported in wine and known to have fruity notes (Fang &
382 Qian, 2005; Yuan & Qian, 2016). It was consistent with the sensory profiling results showing
383 more important fruity and winy attributes in wine formulation. If they are often desired in wines,
384 these fruity/floral notes are less expected in grilled meats where they can be considered as not
385 very congruent and reduce the hedonic score of the formulation. The oregano aromagram was
386 differentiated by high intensity “butter/lactic/cheese” and “cooked vegetable” peaks (>3.5) at
387 550s and 1280s respectively. The caper aromagram was differentiated by its peak at 1000s

388 presenting a “butter/lactic/cheese” note of higher intensity and greater peak width (i.e. longer
389 odor note duration) than the standard.

390 3.3.2. *Identification of the discriminant volatile compounds of the studied* 391 *formulations*

392 In order to highlight which odor zones from the aromagrams permitted to differentiate one
393 formulation from the others, highly discriminant peaks were determined by one-way ANOVA
394 on all the intensity ratings from the eight judges for each odor-active compound. Supplementary
395 data 2 presents the corresponding ANOVAGram. Among these peaks, 10 were found highly
396 discriminant ($P < 0.001$ according to ANOVA) and are presented in Table 3, along with other
397 results from DH-GC-MS/80. Indeed, the data from the olfactometry detector allowed to state
398 the significant peaks for each formulation and the corresponding odor descriptors, while the
399 mass spectrometry data permitted to detect and tentatively identify odor active compounds
400 behind each odor zones highlighted by olfactometry.

401 Table 3 indicated that peaks 3, 7 and 11 permitted to significantly differentiate wine from the
402 standard and from the other formulations. Ethyl isobutyrate (peak 3) and ethyl-2-
403 methylbutanoate (peak 7), markers of wine formulation and both described with fruity
404 descriptors, confirmed the strong contribution of fruity/floral notes previously observed in
405 figure 4. Their presence in wine formulation was coherent with previous studies as they resulted
406 from the fermentation process (Fang & Qian, 2005). Peaks 11 and 18 are still under
407 identification. Mass spectrometry data suggest that peak 11 could be either 4-methyl-
408 cyclohexanone, heptanol or benzaldehyde. As for peak 18, it could be an isomer of
409 dimethyltrisulfide or 2-phenylethanol. Previous works on volatile compounds in wines
410 (Sánchez-Palomo et al., 2005; Fang & Quian, 2006; Girard et al., 2001), showed that, among
411 these possible compounds, 2-phenylethanol, benzaldehyde and heptanol were odor-active
412 compounds found in wine with a floral smell (rose, floral, fruity), a bitter almond odor and a

413 fruity citrus odor respectively. However, in our experiment, the judges described peak 18 with
414 a more “meaty, roasty” smell which therefore did not correspond to the expected odor of 2-
415 phenylethanol. Moreover, peak 11 was not consensually described within the panel as terms as
416 different as mint, sulfur, chemical or fruity were used. In these conditions, a coelution of
417 different chemicals could not be excluded and the heartcutting technique developed in Giri *et*
418 *al.* (2005) would be an interesting option to elucidate the composition of peaks 11 and 18.

419 Data concerning the discriminating peaks for oregano and caper formulations were also
420 informative as they permitted to propose hypotheses to explain their sensorial acceptance by
421 consumers.

422 Oregano and green tea samples were differentiated from the standard and the other formulations
423 by the same significant peaks 9, 16, 17 and 19 as shown in table 3. Based on mass spectrometry
424 results, 2,3-dimethylpyrazine (peak 9) and methyl-2-methyl-3-furyl disulfide (peak 19) were
425 markers of the differentiation of these samples from the standard and other formulations. The
426 odor description of peak 9 as in particular ham and peanut notes was consistent with the data
427 found in literature for 2,3-dimethylpyrazine known to have a meaty, nut-like, coffee aroma.

428 Methyl-2-methyl-3-furyl disulfide (peak 19) was described in particular by the judges as having
429 “burnt paper, fermented grass, vegetable” notes which is consistent with literature data that
430 described it as having sulfurous, cooked and vegetable odors. GC/MS data proposed 3 different
431 possible odorant molecules for peak 16: ethyl heptanoate, 2-nonanone or 3-ethyl-2,5-dimethyl
432 pyrazine. However, different research studies on green tea aromas highlighting the presence of
433 different pyrazines in green tea (Lee et al. 2013; Zhu et al., 2021), lead us to assign peak 16 as
434 being 3-ethyl-2,5-dimethyl pyrazine. As research works on compounds responsible of oregano
435 aromas are mostly focused on terpenes derivatives (De Falco, et al., 2014; Figiel, et al., 2010;
436 Napoli, et al., 2020), it was not possible to check in the literature data whether 3-ethyl-2,5-
437 dimethyl pyrazine is present or not in oregano. In the fast sensory profiling done on the different

438 formulations and the standard, oregano formulation was differentiated from the other
439 formulations and standards by the aromatic plant note. But when looking for the discriminant
440 volatile compounds of oregano formulation evidenced by DH-GC-MS/80, none of the 4
441 discriminant peaks displayed this typical aromatic plant note. However, as evidenced by the
442 hedonic scoring, this formulation is well accepted by consumers as its aromatic note is
443 congruent with meat even if we couldn't identify which chemical compounds were responsible
444 for this characteristic note.

445 Peak 6 found in caper formulation is distinctive from the other formulations (table 3). Based on
446 mass spectrometry data, it was identified as ethyl-2-hydroxypropanoate. Its retention time
447 coincides with the overexpression of the "butter/lactic/cheese" note at 1000-1030s evidenced
448 in caper aromagram compared to the standard (figure 4). This distinctive marker is not very
449 evocative of the caper as described in the work of Sonmezdag et al. (2019) which highlighted
450 strong sensory notes of floral, woody, green, and fresh odors in the study of aroma-active
451 compounds in fresh caper aroma. However, it could with other odor active compounds
452 contribute in mixture to the distinctive note of the caper formulation. This distinctive caper note
453 should not be too pronounced as the results of hedonic scoring and MDS analysis demonstrated
454 the proximity of caper formulation and the standard from a sensorial and sensory acceptability
455 point of view. Peak 18 distinguished caper from oregano and green tea formulations but further
456 interpretation is needed to identify this peak unambiguously. Based on the mass spectrometry
457 data, it could be either an isomer of dimethyltrisulfide or 2-phenylethanol. The work of
458 Conurso *et al.* (2016) on aroma volatiles for the characterization of capers showed that 2-
459 phenylethanol was an odor-active compound of capers. On the contrary, no mention of the
460 presence of an isomer of dimethyltrisulfide in caper could be found in the literature. It would
461 be therefore more probable that peak 18 correspond to 2-phenylethanol. However, as mentioned
462 in the case of wine formulation, the odor descriptors given by the judges, as to know "meaty,

463 roasty” notes, didn’t match those referenced for 2-phenylethanol suggesting a potential
464 coelution issue.

465 This study done on odor-active drivers of the sensorial acceptability differences between
466 mitigating formulations and standard revealed that caper formulation was distinguished from
467 the standard (and the other formulations) by one discriminant peak presenting a
468 “butter/lactic/cheese” note and identified as ethyl-2-hydroxypropanoate. On the contrary,
469 oregano formulation presented strong differences from the standard as it possessed 4
470 discriminant peaks from the standard among which 2,3-dimethylpyrazine and methyl-2-methyl-
471 3-furyl disulfide.

472 Moreover, on a toxicological point of view, the use of dried oregano seems safe. Indeed,
473 culinary oregano contains at most 100mmol of carvacrol (*i.e.* oregano active principle) per 100g
474 of dried leaves (Dragland et al., 2003). So, in the 0.25% (w/w) oregano formulation used in this
475 work, carvacrol is present in lower quantity than the defined tolerable daily intake of 2.5
476 mg/kg/day. Since oregano essential oils are already known for their antibacterial and antifungal
477 properties (Maurya, et al., 2021), the use of oregano formulation to prepare ground beef patties
478 would therefore permit to reduce HAAs formation while limiting bacterial and fungal
479 development.

480

481 **4. Conclusion**

482 In the first part of this work (Meurillon, *et al.*, 2020), it was shown that natural ingredients
483 presenting the best inhibitory activity against HAA formation among the 4 studied were green
484 tea and oregano. The approach proposed in this paper made it possible to select among the 4
485 formulations the ones which were the most acceptable from the sensory point of view: oregano
486 and caper formulations. Based on the present work, oregano would be therefore the best choice
487 as at medium amount (*i.e.* 0.25% mass concentration) it presents an acceptability comparable

488 to that of the target product, as to know cooked ground raw beef patties. This may be due to its
489 relative congruence with cooked meat.

490 Therefore, the combination of the results obtained during these two research works will allow
491 the oregano model to be used to subsequently elucidate the mechanism of inhibition of the
492 formation of HAAs and the impact of this formulation on colorectal carcinogenesis in
493 connection with exposure to HAAs.

494

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499

500 **6. Conflicts of interest**

501 The authors declare that there is no conflict of interest.

502

503 **7. References**

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661 **Figure Captions**

662

663 **Figure 1. Mean hedonic scores (n=59) obtained for the standard and the 4 formulations studied: wine, green**
664 **tea, oregano and caper (2 modalities tested by formulation – marinade either 4 or 6h or blended with 0.25%**
665 **or 0.5% of natural product) by hedonic scoring using a 9-point hedonic scale. Hedonic mean scores were**
666 **compared using Duncan’s test and a letter was attributed to each different group. Error bars represented standard**
667 **error for the means.**

668

669 **Figure 2. Relative positioning of the 4 formulations and 2 standards according to their flavor or odor: 2-D**
670 **multidimensional scaling of the average distance matrix based on 2D flavor dissimilarity assessment (a);**
671 **2-D multidimensional scaling of the average distance matrix based on 2D odor dissimilarity assessment (b)**
672 **according to Bonaïti et al. (2005).**

673

674 **Figure 3. Spider Web of the descriptors giving their average intensity (n=15 assessors) obtained by the 15**
675 **judges during the fast sensory profiling sessions.**

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677 **Figure 4. Mean aromagrams of (A) Standard (B) Oregano formulation (C) Caper formulation calculated**
678 **from the 8 aromagrams obtained by DH-GC-MS/80. The breakdown of the signal into twelve odor classes**
679 **shows the odorant zones belonging to a given olfactory class. These olfactory class are represented by a color**
680 **according to the coding system specified in the box.**

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Table Captions

Table 1. Odor poles and corresponding vocabulary items used by the eight GC-O panelists

Table 2. Comparison of the intensity of the sensory attributes measured by the fast sensory profiling for the 2 standards and 4 formulations according to the 2-way ANOVA

The different intensities of the sensory attributes are given \pm standard deviation and were obtained from naïve judges (n=15) selected for their stronger sensory capacities.

Two-way ANOVA (formulation, judge) was performed on mean attribute intensities according to the model: attribute = formulation + judge.

When significant differences were found ($P < 0.05$), mean attribute intensities were compared using Duncan's multiple comparison test and a letter was attributed to each different group.

with J: judge effect, F: formulation effect, *: P -value < 0.05 ; ** P -value < 0.01 , *** P -value < 0.005

Table 3. Nature of the 10 most significant peaks for the different formulations obtained by coupling olfactometry and mass spectrometry data from DH-GC-MS/80 experiments

From the DH-GC-MS/80 experiments on the different formulations and standards, highly discriminant peaks were determined by one-way ANOVA on all the intensity ratings from the eight judges for each odor-active compound according to the model: attribute = formulation. Among these peaks, table 2 presents the 10 found highly discriminant ($P < 0.001$ according to ANOVA).

The peaks for which the average of intensity rating from the 8 judges is significantly different from 0 are indicated by X in the corresponding formulation column.

with LRI: Linear Retention Indices; S: standard formulation; W: wine formulation; O: oregano formulation; C: caper formulation; T: green tea formulation

^a Chemical name resolved by DH-GC-MS/80 experiments using mass spectrometry data and odor descriptors for a given peak

^b Odor descriptor given by the 8 judges during DH-GC-MS/80 experiments