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Performance testing of new artificial olfactory reference materials in virgin olive oil sensory assessment

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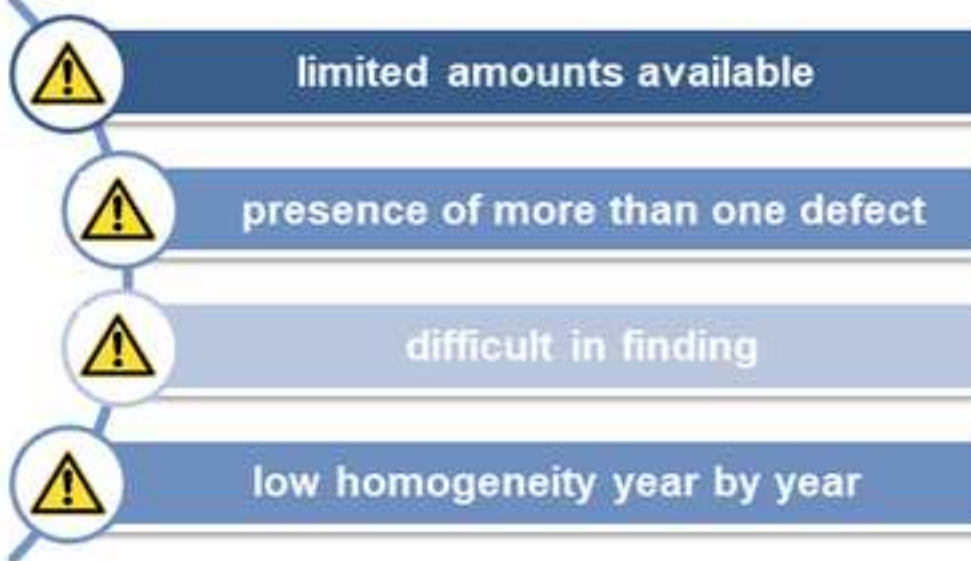
Highlights

- The use of RMs for sensory evaluation of VOO is fundamental to ensure the performance of panel test
- Applications of RMs: selection, training, monitoring of tasters, panel leaders, and trainers
- Advantages of RMs: reproducibility over time, possibility of purchase

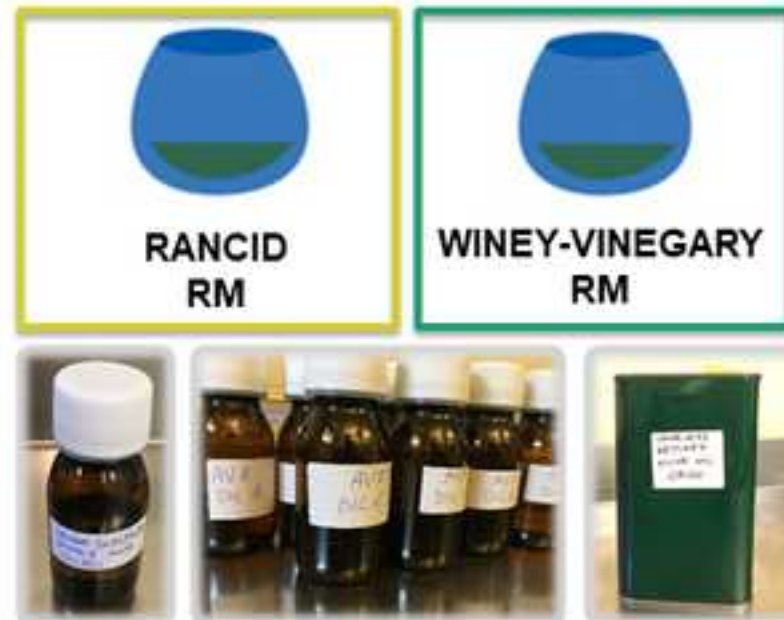
Abstract: The use of reference materials for sensory evaluation of virgin olive oils by tasters, panel leaders and trainers represents a fundamental tool to ensure the performance of panel test performance in meeting regulatory demands and protect the quality of the product. Herein, a procedure for sensory certification of two, recently formulated, artificial olfactory reference materials (RMs) for winey-vinegary and rancid defects of virgin olive oil is presented. A technical protocol, consisting of different steps aimed at evaluating the representativeness of the sensory defect, determining the detection threshold and estimating stability over time, was applied by six sensory panels involved in the H2020 OLEUM project. Considering the homogeneity of the results provided by panels, the procedure can be considered reliable and able to characterize aroma reference materials that appear to be suitable for their intended use according to the international standards.

The possible introduction and use of these new RMs to support selection and training of tasters and increase the control and proficiency of sensory panels can improve the effectiveness of the method by offering advantages such as the reproducibility over time and/or the possibility of purchase or self-preparation, thereby overcoming the lack of availability and year-by-year variation of the current oils used as references.

*RM*s for VOO sensory analysis from natural matrix



*Artificial olfactory RM*s for VOO sensory analysis



Performance testing of new artificial olfactory reference materials in virgin olive oil sensory assessment

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1 **Performance testing of new artificial olfactory reference materials in virgin olive oil sensory** 2 **assessment**

3

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5 leaders and trainers represents a fundamental tool to ensure the performance of panel test performance
6 in meeting regulatory demands and protect the quality of the product. Herein, a procedure for sensory
7 certification of two, recently formulated, artificial olfactory reference materials (RMs) for winey-
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9 different steps aimed at evaluating the representativeness of the sensory defect, determining the
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16 by offering advantages such as the reproducibility over time and/or the possibility of purchase or self-
17 preparation, thereby overcoming the lack of availability and year-by-year variation of the current oils
18 used as references.

19

20 **Keywords:** virgin olive oil; reference materials; winey-vinegary defect; rancid defect; sensory
21 assessment; volatile compounds.

22

23 **Implications for gastronomy**

24 Virgin olive oil (VOO) has become an essential ingredient not only in Mediterranean gastronomy,
25 but also in international cuisine, with multiple uses, as a fresh ingredient or in diverse culinary
26 practices (Brkić Bubola et al., 2020; Rinaldi de Alvarenga et al., 2019). Nowadays, the use of VOO

27 is promoted among professional and amateur cooks due to its known health benefits and unique
28 sensory characteristics (Karković Marković et al., 2019; Celano et al., 2019). Aroma and taste of
29 VOOs are the most important key intrinsic factors for the consumers' perception of oil sensory quality
30 (Lukić et al., 2018) and these characteristics are evaluated by the application of an official method,
31 called panel test, performed by a group of trained testers (EEC Reg. 2568/91). Panel test establishes
32 the required sensory properties (the main positive and negative attributes) for each of the quality
33 categories, extra virgin, virgin and lampante olive oil, the first two being those accessed by consumers
34 for the different culinary uses. The correct application of panel test aims to guarantee that the declared
35 category has the expected sensory properties avoiding the case of marketing of lower quality products
36 labelled as extra virgin olive oil, which would affect the consumer expectation. The development of
37 RMs for panel training and control has been identified as one of the most important needs. The
38 adoption of RMs by associations that organize dissemination activities as sensory courses may also
39 increase knowledge and awareness of end-users as restaurateurs and chefs on the sensory
40 characteristics of VOOs.

41

42 **Abbreviations**

43 Reference material, RM; protected designation of origin, PDO; International olive council, IOC; extra
44 virgin olive oil, EVOO; virgin olive oil, VOO; lampante olive oil, LOO; certified reference material,
45 CRM; odorless refined olive oil, OROO.

46

47 **1. Introduction**

48 Sensory evaluation mainly focuses on measuring the responses of people to the sensory properties of
49 foods (Sidel and Stone, 2005); this discipline is recognized as a scientific field and there has been
50 growing interest in its application to product development and quality control. The verification of a
51 sensory profile compliance with specific standards is a pressing need for the certification of food
52 products, especially for foods and beverages that possess specific qualities and an extremely strong

53 link to the territory such as those with protected designation of origin (PDO) (EU Reg. 1151/2012).
54 For these products, the certification of the sensory characteristics is voluntary, but for some quality
55 control procedures it is mandatory as in the case of virgin olive oil (VOO) (EEC Reg. 2568/91;
56 Barbieri et al., 2018).

57 The 2019 Report on the EU Food Fraud Network activities provided information on non-compliances
58 and potential intentional violations of the EU agrifood chain legislation, highlighting “fats and oils”
59 as the most notified product category placing “olive oil” as the most notified product in the system
60 (EC 2019). Considering the importance of VOO for the economy of Mediterranean countries, its
61 nutritional and health properties, and the protection of its quality and authenticity from illegal
62 practices implemented for profit, these aspect represent enormous challenges for both the scientific
63 community and those involved in the olive oil supply chain (Conte et al., 2020).

64 In order to protect the final consumer, the International Olive Council (IOC) and the European Union
65 (EU) regulated the definition of VOO and its commercial categories by introducing chemical and
66 sensory parameters to verify the quality of the product and its authenticity in terms of genuineness or
67 purity. Since 1991, the evaluation of the sensory characteristics of VOO has been included among
68 the quality parameters by application of the panel test method (EEC Reg. 2568/91) provided by the
69 International Olive Council (IOOC/T.20/Doc.no.3, 1987). This approach is based on the evaluation
70 of a group of tasters (panel) that, after being selected and constantly trained, must be monitored for
71 correct product classification (extra virgin olive oil, EVOO; virgin olive oil, VOO; lampante olive oil,
72 LOO), and for the recognition of the intensity of sensory attributes (positives and negatives).

73 Different documents issued by the IOC describe in detail the equipment, methodology to be applied
74 and standards that need to be adopted with extreme rigor to guarantee the reliability of the panel test
75 (Barbieri et al., 2020). One of these documents reports on the rules and practices to be applied during
76 the selection, training, and monitoring of VOO tasters, panel leaders, and trainers that are fundamental
77 to enhance the panel’s proficiency in recognizing, identifying, and quantifying sensory attributes
78 (IOC/T.20/Doc. no.14/Rev.6, 2020). People who intend to become tasters of VOO are initially

79 selected through the application of tests to verify their sensitivity to odors and tastes and
80 discriminatory skills (e.g. determination of the detection threshold of panellist candidates and
81 application of the intensity rating method); subsequently, during the training phase, tasters needs to
82 become familiar with specific sensory methodology and sensory attributes (practical trials for
83 recognizing by smell and taste the characteristic negative and positive attributes of VOO and their
84 intensities; use the official profile sheet for classification of the various categories of VOO; tasting
85 and becoming familiar with sensory characteristics of national/international olive varieties). Once the
86 panel has been set up, its maintenance is performed through continuous training and by the application
87 of quality control procedures, and verification of the sensory acuity of tasters and panel performance.
88 Moreover, each year, all panels must undergo a proficiency test in which several reference samples
89 must be assessed to verify the reliability of the results from different panels and to harmonize their
90 perception criteria.

91 For all these procedures, the use of reference materials (RM) for which definitive numerical values
92 can be associated with different sensory attributes is of absolute necessity to calibrate the
93 measurement process, help assessors in memorization of sensory stimuli, and compare their judgment
94 with “assigned values” and thus improve their individual abilities. RMs are commonly used to support
95 measurement procedures (e.g. method validation, calibration, training, quality control, and quality
96 assurance purposes) and may enable the expression of functional properties in arbitrary units (EA,
97 2003; Karambelkar, 2018).

98 Thus, in the particular case of sensory analysis, the lack of a proper RM would introduce errors in
99 measurements and would pose a difficulty in the harmonization of panels and panellists in their
100 responses (Aparicio-Ruiz et al., 2019; Aparicio-Ruiz et al., 2020).

101 Two classes of materials are recognized by the ISO, namely certified RMs (CRMs) and RMs. CRMs
102 must be accompanied by a certificate issued by a certifying body that attests the accurate procedure
103 for obtaining the proper values that are expressed (traceability) with an uncertainty at a stated level

104 of confidence. RMs are materials or substances whose property values are sufficiently homogeneous,
105 stable, and well established for its intended use in a measurement process (ISO, 1992; ISO, 2000).

106 Taking into account these required properties, some of the key issues that need to be considered in
107 the preparation of RMs are the appropriateness of selected materials, homogeneity in testing,
108 preparation (including packaging), and stability during distribution and storage (EA, 2003;
109 Karambelkar, 2018).

110 For sensory analysis of VOOs, at present only RMs from a natural matrix (VOOs that are used as
111 reference samples of sensory defects provided by IOC, samples from proficiency tests, or certified
112 by at least three accredited sensory panels) are available for training of sensory panels (Conte et al.,
113 2020); they are effective in resembling real samples, although they have some limitations in their use
114 over time (e.g. limited availability, difficult to obtain, low homogeneity year by year) (Aparicio-Ruiz
115 et al., 2020).

116 Despite the extensive literature on the application of sensory analysis for VOO quality control, only
117 a few studies concerning the use and assessment of RMs for VOO sensory analysis have been carried
118 out. Some researchers have recently proposed the use of fingerprinting chromatographic signal as
119 alternative technique to sensory analysis to monitor the stability RMs for VOO over time (Valverde-
120 Som et al., 2018a; Valverde-Som et al., 2018b) and to assess their homogeneity (Ortega-Gavilán et
121 al., 2020) by evaluating the similarity between fingerprint signals on the volatile fraction of samples.

122 In a newly published study, carried out within the framework of the OLEUM project (Horizon 2020,
123 Grant Agreement No. 635690), a strategy for designing RMs as supporting tools for panel training
124 was presented and the optimization process to obtain the best formulation for rancid and winey-
125 vinegary defects has been illustrated (Aparicio-Ruiz et al., 2020). Representativeness, aroma
126 persistence, and simplicity in composition were the main criteria applied to obtain a formulation that,
127 with an open-source composition, should offer several advantages including reproducible
128 manufacturing of RMs and the possibility of in-house preparation or purchase of RM that are already
129 prepared.

130 The present work represents the continuation of the activities carried out by Aparicio-Ruiz and co-
131 authors (Aparicio-Ruiz et al., 2020), giving an overview of the practical application and test
132 certification of two new formulated aroma RMs by sensory analysis. Specifically, a protocol for the
133 suitability of RMs has been defined, shared, and applied by six sensory panels involved in the project;
134 it consisted of three steps: i) evaluation of representativeness of RMs (compared to actual samples)
135 and of its intensity values; ii) determination of the panels' detection threshold and; iii) evaluation of
136 long-term stability. To the best of our knowledge, this represents the first attempt to certify RMs for
137 VOO by sensory analysis.

138

139 **2. Materials and Methods**

140 *2.1 RMs for VOO and sensory panels*

141 Six sensory panels (EUROFINS from Germany; IPTPO from Croatia; ITERG from France; UNIBO
142 from Italy; ZRS from Slovenia and UZZK from Turkey), recognized by national (e.g. Italian Ministry
143 of Agricultural, Food and Forestry Policies) or/and international (e.g. International Olive Council)
144 bodies, were involved in sensory activities as partners in the OLEUM project. The UNIBO panel was
145 responsible for the coordination of sensory activities and for elaboration of sensory data. The RMs of
146 VOO used in the present study were formulated for resembling the aroma of winey-vinegary and
147 rancid defects using specific mixtures of volatile molecules in selected concentrations, as described
148 by Aparicio-Ruiz et al. 2020.

149 Stock solutions of the selected formulations of RMs (winey-vinegary, coded as AV8 and rancid,
150 coded as R7) were prepared, divided into aliquots, and sent to the other panels by UNIBO together
151 with a kit of materials and documents to proceed in sensory evaluation (as detailed in Table 1). In
152 order to preserve quality and sensory properties, storage of RMs and VOO samples at 10-12°C in
153 dark conditions and reconditioning at room temperature for 6-8 hours before analysis was
154 recommended.

155

156 Table 1. Documents and materials provided to panels for sensory evaluation of RMs.

Kit for sensory evaluation of RMs
<ul style="list-style-type: none">• Technical protocol for sensory evaluation (only by smell) of RMs including details for preparation of the RM sensory session preparation• RM of winey-vinegary stock solution (code AV8, 50 mL)• RM of rancid stock solution (code R7, 50 mL)• Actual sample of VOO with an intensity of winey-vinegary defect of 1.7¹ (code IT_35)• Actual sample of VOO with an intensity of rancid defect of 2.3¹ (code IP_45)• Odorless refined olive oil (OROO) needed for dilution of RM samples (600 mL)• Empty containers suitable to arrange and store stock solutions and dilutions of RMs• RM evaluation sheet to be used by both panel leaders and assessors

157 ¹Mean of median values from six panels (Barbieri et al., 2020).

158

159 *2.2 Technical protocol for sensory evaluation of RMs*

160 The technical protocol for sensory evaluation of RMs provided to each panel required, as a first step,
161 the evaluation of the representativeness of the two selected formulations (Aparicio-Ruiz et al., 2020)
162 in terms of similarity when compared with the corresponding odor characteristics of a real defect,
163 odor intensity, and odor persistence.

164 Specifically, each assessor was asked to evaluate the adequacy of stock solution of each RMs (AV8
165 and R7), to provide their intensity by comparing them with real samples (IT_35 for winey and IP_45
166 for rancid), and to report any comments. Each panel leader was asked to collect sensory data provided
167 by the panel leader and at least 8 assessors (at least 9 in total) and to send them to the UNIBO panel
168 for elaboration.

169 In the second part of protocol, the determination of the detection threshold of each panel (panel leader
170 + at least 8 assessors) was required. For each stock solution of RMs (AV8 and R7), successive
171 dilutions were prepared by each panel leader by applying the same methodology described by the

172 IOC for determination of detection thresholds of the group of candidates for characteristic VOO
173 attributes (IOC/T.20/Doc. no.14/Rev.6, 2020).

174 The paired difference tests, where the tasters are not asked to recognize the attribute but only to state
175 if they detect a stimulus, was applied for each of the two RMs (AV8 and R7) and its dilutions in
176 odorless refined olive oil (OROO). In addition, assessors were also asked to quantify the intensity (on
177 a linear scale) of each dilution.

178 Specifically, each panel leader prepared a series of samples for each stock solution (AV8 and R7) in
179 descending concentrations (dilutions 1:1 v/v) by making successive dilutions in OROO. The series
180 were considered complete when no difference could be detected (by each panel leader) between two
181 successive dilutions and the OROO. The panel leader then chose the seven dilutions prior to these
182 last two. Paired difference tests by smelling were carried out; specifically, each assessor (also the
183 panel leader) was presented with up to a total of 8 pairs of samples, randomly presented and in
184 successively independent tests (the pairs comprise one of each seven samples chosen and OROO,
185 plus one pair of OROO). In each test, candidates were asked if the two samples (using different codes
186 for each dilution) were identical or different and to report their intensity.

187 The detection threshold of a candidate was the dilution that he or she found to be different from
188 OROO, while this was not the case with the next more diluted samples.

189 The test was realized by tasters one by one and the use of device for heating samples ($28 \pm 2^\circ\text{C}$),
190 standardized glasses for VOO tasting (IOC/T.20/Doc.no.5/Rev.1, 2007; IOC/T.20/Doc.no. 6/rev.1,
191 2007) and covers for glasses as well as a pause of several minutes between the tests, were
192 recommended. Each panel leader was asked to collect sensory data (panel leader + at least 8 assessors)
193 and to send them to the UNIBO panel for elaboration. After the comparison tests carried out, each
194 panel leader sent the results to UNIBO who processed the data, noting the correct answers for each
195 concentration and expressing them in percentages. The detection threshold of the panel corresponded
196 to the percentage dilution of RM at which 75% of assessors answered correctly (calculated by
197 interpolation) (IOC/T.20/Doc. no.14/Rev.6, 2020).

198 Stability evaluation was also necessary to provide important information on possible modifications
199 of the aroma during the storage period and estimate the best before date of each RM. For this reason,
200 as the third part of the technical protocol for sensory evaluation of RMs, sensory evaluation by
201 smelling the 7 dilutions of each RM (previously chosen by panel leader) and the two stock solutions
202 (AV8 and R7) were also repeated after 3 and 6 months.

203 To protect RMs (stock solutions and its dilutions) as much as possible from light and heat, avoiding
204 contamination from extraneous material, storage at a temperature of 10-12°C in the dark was
205 recommended during the stability study, avoiding contamination from extraneous material
206 throughout this period. Each panel leader was asked to collect sensory data (panel leader + at least 8
207 assessors) and to send them to the UNIBO panel.

208 Analysis of variance (ANOVA) followed by Fishers LSD post-hoc test ($p < 0.05$) was carried out to
209 highlight possible significant differences between samples.

210

211 **3. Results and discussion**

212 *3.1 Evaluation of representativeness of RMs*

213 Data from the evaluation of the representativeness of the two RMs (AV8 and R7) confirmed the
214 preliminary results obtained by Aparicio-Ruiz et al. 2020. Specifically, in the case of winey-vinegary
215 RM, 97% of assessors (of a total of 62) considered the RM stock solution to be useful in assigning an
216 intensity value of 8.0 that, in some cases, was considered too high. For the rancid RM, 82% of
217 assessors (51 of 62) considered the RM useful and assigned an intensity value of 8.0, but some tasters
218 noted differences from the real defect due to the presence of other secondary notes such as fried oil
219 and/or bedbug. Results and comments provided by tasters are reported in Table 2.

220

221 Table 2. Results from the first part of the protocol on the practical application of the stock solution of
222 each RM: winey-vinegary (AV8) and rancid (R7).

RM	% usefulness	Intensity (Me)	s*	CVr%	C.I upper C.I. lower	Comments
Winey- vinegary	97	8.0	0.3	3.7	8.6 7.4	Too intense ¹
Rancid	82	8.0	0.2	3.0	8.4 7.5	Presence of other odours than rancid (e.g. bedbug, fried oil) ²

223 Note: ¹comment provided by 2 of 6 panels; ²comment provided by 1 of 6 panels. Data were expressed
224 as median (Me) of 62 testers belonging to 6 panels, robust standard deviation (s*), robust coefficient
225 of variation % (CVr%), confidence intervals (C.I.) of the median at 95%.

226

227 3.2 Determination of detection threshold

228 The second part of the protocol aimed to determine the average threshold perception of the six panels
229 for each RM, but also to create a range of RM concentrations that would be useful to identify those
230 close to the intensities that, for sensory defects, define the two commercial categories ($VOO \leq 3.5$,
231 $LOO > 3.5$). Figures 1 and 2 show the values of the detection thresholds and intensity of dilutions
232 provided by the six panels for the RMs of winey-vinegary and rancid defects (AV8 and R7).

233 Sensory data showed that the detection threshold of the six panels for the winey-vinegary RM fell
234 between dilutions no. 8 and no. 9 (0.4% and 0.2% dilutions), which corresponded to a perceived
235 intensity between 0.3 and 1.0. In the case of rancid RM, the detection threshold was set between
236 dilutions no. 9 and no. 10 (0.2% and 0.1% dilutions) which corresponded to a perceived intensity
237 between 0.3 and 1.1.

238

239 3.3 Evaluation of long term stability.

240 Finally, in the third part of technical protocol for sensory evaluation of RMs, tasters from six panels
 241 were asked to re-evaluate the stock solutions and the 7 dilutions of each RM after 3 (time 1) and 6
 242 (time 2) months from their preparation. In order to compare results between the tested time of storage
 243 highlighting possible significant differences, data were expressed as mean values and analysis of
 244 variance was performed (Table 3).

245

246 Table 3. Stability evaluation of RMs at time 0 (t0), after 3 (t1) and after 6 (t2) months of storage.

Winey- vinegary RM	Stock solution	Dil.3	Dil.4	Dil.5	Dil.6	Dil.7	Dil.8	Dil.9
Intensity_t0	7.7 ^a	4.7 ^a	4.0 ^a	2.5 ^a	1.9 ^a	1.6 ^a	1.1 ^a	0.4 ^a
CV%	18	26	21	38	54	53	45	141
Intensity_t1	7.4 ^a	4.5 ^a	3.4 ^a	2.4 ^a	2.2 ^a	1.7 ^a	1.2 ^a	0.8 ^a
CV%	18	42	36	39	39	42	43	78
Intensity_t2	7.4 ^a	4.0 ^a	3.2 ^a	2.6 ^a	2.3 ^a	1.8 ^a	1.4 ^a	0.8 ^a
CV%	19	5	24	30	46	52	57	82
Rancid RM	Stock solution	Dil.4	Dil.5	Dil.6	Dil.7	Dil.8	Dil.9	Dil.10
Intensity_t0	7.9 ^a	4.5 ^a	4.0 ^a	3.0 ^a	2.5 ^a	1.4 ^a	1.1 ^a	0.4 ^a
CV%	12	25	21	32	44	75	73	125
Intensity_t1	8.3 ^a	4.9 ^a	4.6 ^a	3.8 ^a	3.2 ^a	2.1 ^a	1.6 ^a	1.1 ^a
CV%	13	34	43	48	54	39	43	101
Intensity_t2	8.6 ^a	5.7 ^a	4.7 ^a	3.6 ^a	2.9 ^a	2.0 ^a	1.5 ^a	0.9 ^a
CV%	9	10	13	28	34	48	50	102

The data presented are related to the intensity expressed as mean values (median from 6 panels) of the stock solution and of the 7 dilutions of RMs of winey-vinegary and rancid defects. CVr%, robust

coefficient of variation %. Values labeled with a different letter, within one RM and same column are statistically different (Fishers LSD post-hoc test, $p < 0.05$).

247 The results showed that there were no significant differences for either RM (winey-vinegary and
248 rancid) at different concentrations, passing from 0 to 3 or 6 months.

249 As reported by Karambelkar, 2018 “stability is the characteristic of a reference material, when stored
250 under specified conditions, to maintain a specified property value within specified limits for a
251 specified period of time”. For this purpose, the evaluation carried out can indicate the interval of
252 intensities for each of the two RMs, at one selected dilution, under the tested conditions. For example,
253 considering the possible utility of these RMs in training of assessors to define the intensity value of
254 the specific defect on a 10 cm linear scale, it could be very useful to point out the attention to intervals
255 of intensities closer to borderline (3.5 as median value) between the VOO and LOO quality grades.
256 For this reason, it is possible to observe the same data expressed as median values of stock solutions
257 and dilutions of RMs at 0, 3 and 6 months according to the official method (Table 4).

258

259 Table 4. Stability evaluation of RMs at time 0 (t0), after 3 (t1) and after 6 (t2) months of storage.

Winey- vinegary RM	Stock solution	Dil.3	Dil.4	Dil.5	Dil.6	Dil.7	Dil.8	Dil.9
Intensity_t0	8.0	4.5	3.9	2.5	1.9	1.6	1.0	0.3
CVr%	3.7	6.1	6.8	12.7	12.4	15.6	12.9	43.1
Intensity_t1	7.5	4.5	3.5	2.1	2.4	2.0	1.2	0.8
CVr%	3.0	10.6	7.4	12.5	8.8	8.4	13.1	20.3
Intensity_t2	8.1	4.1	3.5	2.9	2.5	1.6	1.4	0.5
CVr%	3.0	5.7	4.6	8.1	9.4	12.9	13.8	38.6

Rancid RM	Stock solution	Dil.4	Dil.5	Dil.6	Dil.7	Dil.8	Dil.9	Dil.10
Intensity_t0	8.0	4.5	4.0	3.0	2.3	1.5	1.1	0.3
CVr%	3.0	4.4	5.0	6.7	8.6	11.4	7.8	39.2
Intensity_t1	8.0	4.8	5.0	3.7	3.5	2.2	1.7	1.0
CVr%	2.3	6.9	6.9	8.4	8.8	12.3	11.5	24.5
Intensity_t2	8.5	6.0	4.9	3.7	3.2	2.2	1.5	1.0
CVr%	1.6	2.9	4.3	8.3	7.8	9.4	12.0	27.6

260 The data presented are related to the intensity expressed as median values (62 assessors) of the stock
261 solution and of the 7 dilutions of winey-vinegary and rancid defects. CVr%, robust coefficient of
262 variation %.

263

264 The CVr% values exceed the limit foreseen only in the cases of the dilution no. 9 (RM of winey-
265 vinegary) and dilution no. 9 (RM of rancid), which correspond to the lowest intensities; when the
266 median is very close to zero as for very mild perceptions, CVr % greatly increases, often overcoming
267 the limit of 20.0 (Amelio, 2019).

268

269 **4. Conclusions**

270 The proposed RMs were judged as representative of real samples of defected VOOs and exhibited
271 suitable long-term stability. Furthermore, determination of the detection threshold of panels was
272 effective in establishing the minimum perception limit, but also to certify different intensities of the
273 proposed dilutions of RMs. In this way, it is possible to expand the range of activities in which these
274 RMs can be applied for the selection, training, and monitoring of VOO tasters, panel leaders, and
275 trainers. The possibility to prepare and use the indentical RMs in each sensory laboratory, strictly
276 following an detailed open access protocol, could increase the homogeneity of results from panels.
277 On the other hand, for labs that prefer to purchase RMs it is desirable that the present paper and the

278 results of inter-laboratory tests (validation) which are still in progress, involving sensory panels from
279 different countries, will promote their production and availability on the market. RMs are definitely
280 be of interest for a large number of official and professional sensory panels for VOO evaluation
281 worldwide (as well as associations that organize sensory courses to select and train new assessors).
282 The introduction of new RMs does not have the aim of replacing those already in use, but rather to
283 offer an alternative to single and “pure” defects, with training purposes.
284 In addition, future studies should be addressed to the development of RMs for other sensory defects,
285 or to the application of other formulation methods such as fermentative and biotechnological
286 production.

287

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313

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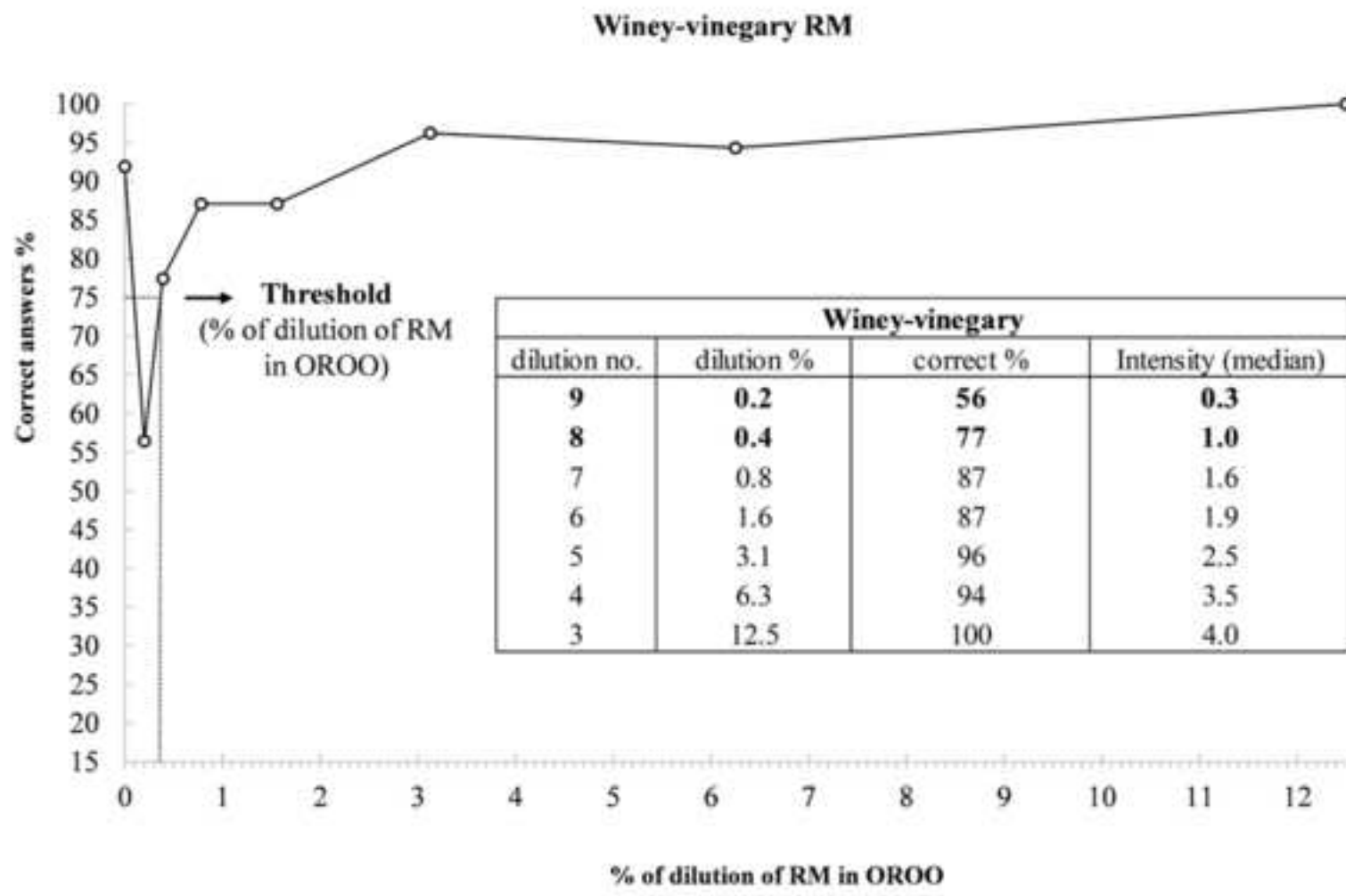
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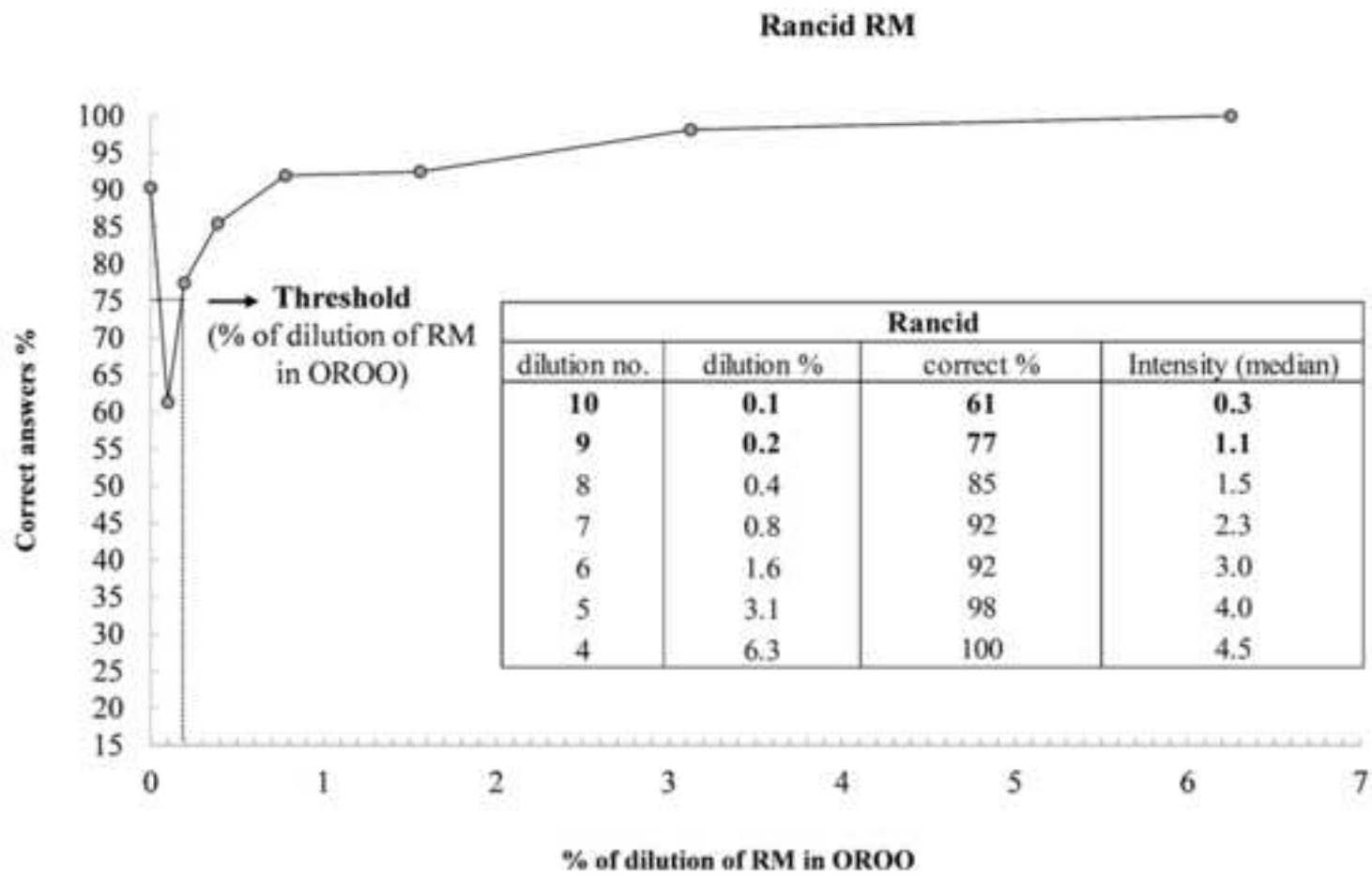
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Tables

Table 1. Documents and materials provided to panels for sensory evaluation of RMs.

Kit for sensory evaluation of RMs
<ul style="list-style-type: none">• Technical protocol for sensory evaluation (only by smell) of RMs including details for preparation of the RM sensory session preparation• RM of winey-vinegary stock solution (code AV8, 50 mL)• RM of rancid stock solution (code R7, 50 mL)• Actual sample of VOO with an intensity of winey-vinegary defect of 1.7¹ (code IT_35)• Actual sample of VOO with an intensity of rancid defect of 2.3¹ (code IP_45)• Odorless refined olive oil (OROO) needed for dilution of RM samples (600 mL)• Empty containers suitable to arrange and store stock solutions and dilutions of RMs• RM evaluation sheet to be used by both panel leaders and assessors

¹Mean of median values from six panels (Barbieri et al., 2020).

Table 2. Results from the first part of the protocol on the practical application of the stock solution of each RM: winey-vinegary (AV8) and rancid (R7).

RM	% usefulness	Intensity (Me)	s^*	CVr%	C.I upper C.I. lower	Comments
Winey-vinegary	97	8.0	0.3	3.7	8.6 7.4	Too intense ¹
Rancid	82	8.0	0.2	3.0	8.4 7.5	Presence of other odours than rancid (e.g. bedbug, fried oil) ²

Note: ¹comment provided by 2 of 6 panels; ²comment provided by 1 of 6 panels. Data were expressed as median (Me) of 62 testers belonging to 6 panels, robust standard deviation (s^*), robust coefficient of variation % (CVr%), confidence intervals (C.I.) of the median at 95%.

Table 3. Stability evaluation of RMs at time 0 (t0), after 3 (t1) and after 6 (t2) months of storage.

Winey- vinegary RM	Stock solution	Dil.3	Dil.4	Dil.5	Dil.6	Dil.7	Dil.8	Dil.9
Intensity_t0	7.7 ^a	4.7 ^a	4.0 ^a	2.5 ^a	1.9 ^a	1.6 ^a	1.1 ^a	0.4 ^a
CV%	18	26	21	38	54	53	45	141
Intensity_t1	7.4 ^a	4.5 ^a	3.4 ^a	2.4 ^a	2.2 ^a	1.7 ^a	1.2 ^a	0.8 ^a
CV%	18	42	36	39	39	42	43	78
Intensity_t2	7.4 ^a	4.0 ^a	3.2 ^a	2.6 ^a	2.3 ^a	1.8 ^a	1.4 ^a	0.8 ^a
CV%	19	5	24	30	46	52	57	82
Rancid RM	Stock solution	Dil.4	Dil.5	Dil.6	Dil.7	Dil.8	Dil.9	Dil.10
Intensity_t0	7.9 ^a	4.5 ^a	4.0 ^a	3.0 ^a	2.5 ^a	1.4 ^a	1.1 ^a	0.4 ^a
CV%	12	25	21	32	44	75	73	125
Intensity_t1	8.3 ^a	4.9 ^a	4.6 ^a	3.8 ^a	3.2 ^a	2.1 ^a	1.6 ^a	1.1 ^a
CV%	13	34	43	48	54	39	43	101
Intensity_t2	8.6 ^a	5.7 ^a	4.7 ^a	3.6 ^a	2.9 ^a	2.0 ^a	1.5 ^a	0.9 ^a
CV%	9	10	13	28	34	48	50	102

The data presented are related to the intensity expressed as mean values (median from 6 panels) of the stock solution and of the 7 dilutions of RMs of winey-vinegary and rancid defects. CVr%, robust coefficient of variation %. Values labeled with a different letter, within one RM and same column are statistically different (Fishers LSD post-hoc test, $p < 0.05$).

Table 4. Stability evaluation of RMs at time 0 (t0), after 3 (t1) and after 6 (t2) months of storage.

Winey- vinegary RM	Stock solution	Dil.3	Dil.4	Dil.5	Dil.6	Dil.7	Dil.8	Dil.9
Intensity_t0	8.0	4.5	3.9	2.5	1.9	1.6	1.0	0.3
CVr%	3.7	6.1	6.8	12.7	12.4	15.6	12.9	43.1
Intensity_t1	7.5	4.5	3.5	2.1	2.4	2.0	1.2	0.8
CVr%	3.0	10.6	7.4	12.5	8.8	8.4	13.1	20.3
Intensity_t2	8.1	4.1	3.5	2.9	2.5	1.6	1.4	0.5
CVr%	3.0	5.7	4.6	8.1	9.4	12.9	13.8	38.6
Rancid RM	Stock solution	Dil.4	Dil.5	Dil.6	Dil.7	Dil.8	Dil.9	Dil.10
Intensity_t0	8.0	4.5	4.0	3.0	2.3	1.5	1.1	0.3
CVr%	3.0	4.4	5.0	6.7	8.6	11.4	7.8	39.2
Intensity_t1	8.0	4.8	5.0	3.7	3.5	2.2	1.7	1.0
CVr%	2.3	6.9	6.9	8.4	8.8	12.3	11.5	24.5
Intensity_t2	8.5	6.0	4.9	3.7	3.2	2.2	1.5	1.0
CVr%	1.6	2.9	4.3	8.3	7.8	9.4	12.0	27.6

The data presented are related to the intensity expressed as median values (62 assessors) of the stock solution and of the 7 dilutions of winey-vinegary and rancid defects. CVr%, robust coefficient of variation %.

Implications for gastronomy

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2 Virgin olive oil (VOO) has become an essential ingredient not only in Mediterranean gastronomy,
3
4 but also in international cuisine, with multiple uses, as a fresh ingredient or in diverse culinary
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6 practices (Brkić Bubola et al., 2020; Rinaldi de Alvarenga et al., 2019). Nowadays, the use of VOO
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8 is promoted among professional and amateur cooks due to its known health benefits and unique
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10 sensory characteristics (Karković Marković et al., 2019; Celano et al., 2019). Aroma and taste of
11
12 VOOs are the most important key intrinsic factors for the consumers' perception of oil sensory quality
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14 (Lukić et al., 2018) and these characteristics are evaluated by the application of an official method,
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16 called panel test, performed by a group of trained testers (EEC Reg. 2568/91). Panel test establishes
17
18 the required sensory properties (the main positive and negative attributes) for each of the quality
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20 categories, extra virgin, virgin and lampante olive oil, the first two being those accessed by consumers
21
22 for the different culinary uses. The correct application of panel test aims to guarantee that the declared
23
24 category has the expected sensory properties avoiding the case of marketing of lower quality products
25
26 labelled as extra virgin olive oil, which would affect the consumer expectation. The development of
27
28 RMs for panel training and control has been identified as one of the most important needs. The
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30 adoption of RMs by associations that organize dissemination activities as sensory courses may also
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32 increase knowledge and awareness of end-users as restaurateurs and chefs on the sensory
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34 characteristics of VOOs.
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Performance testing of new artificial olfactory reference materials in virgin olive oil sensory assessment

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