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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.

Graphical Abstract

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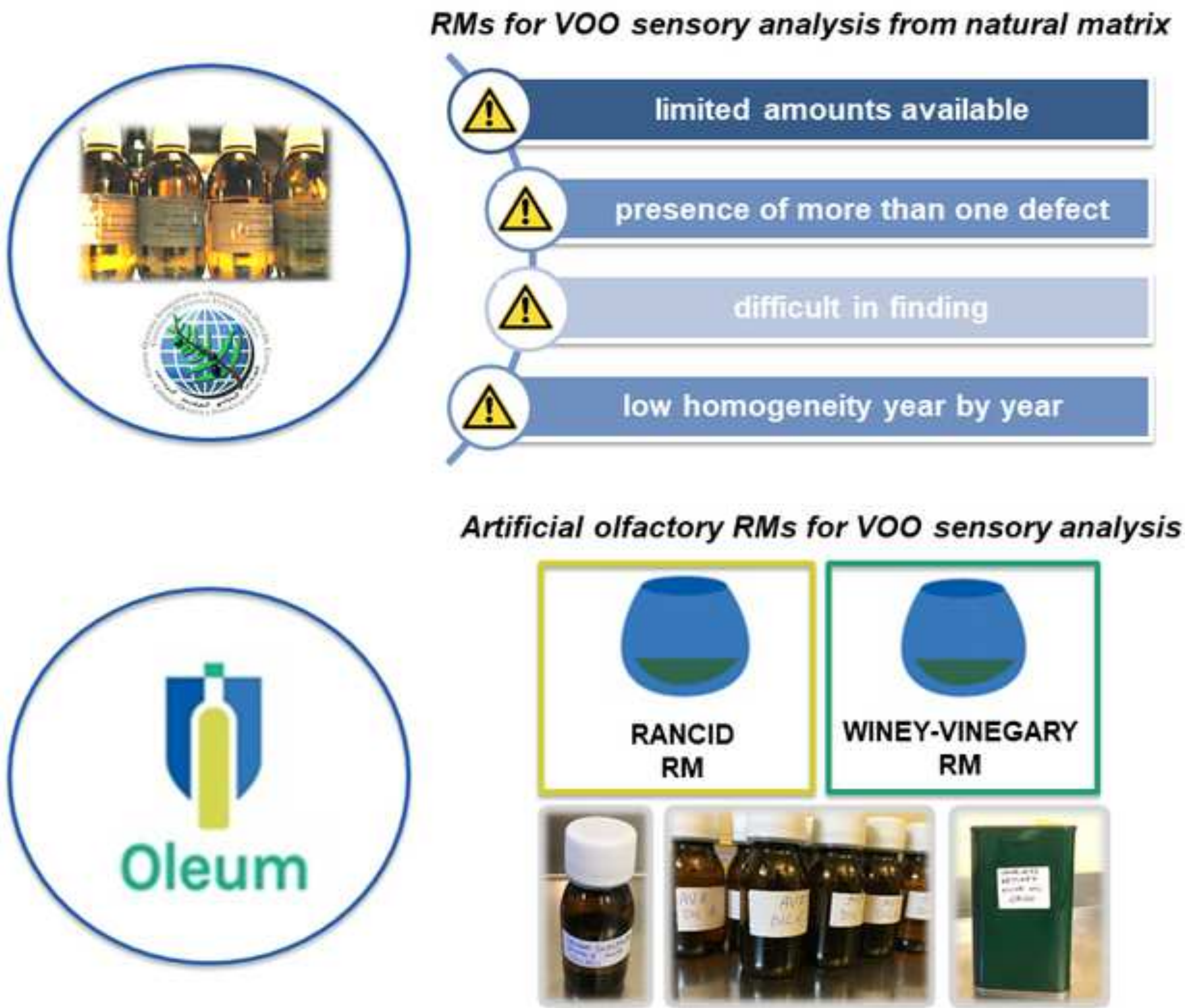

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Performance testing of new artificial olfactory reference materials in virgin olive oil sensory assessment

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.

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

Implications for gastronomy

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

Abbreviations

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

1. Introduction

Sensory evaluation mainly focuses on measuring the responses of people to the sensory properties of foods (Sidel and Stone, 2005); this discipline is recognized as a scientific field and there has been growing interest in its application to product development and quality control. The verification of a sensory profile compliance with specific standards is a pressing need for the certification of food products, especially for foods and beverages that possess specific qualities and an extremely strong link to the territory such as those with protected designation of origin (PDO) (EU Reg. 1151/2012). For these products, the certification of the sensory characteristics is voluntary, but for some quality control procedures it is mandatory as in the case of virgin olive oil (VOO) (EEC Reg. 2568/91; Barbieri et al., 2018).

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

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

Different documents issued by the IOC describe in detail the equipment, methodology to be applied and standards that need to be adopted with extreme rigor to guarantee the reliability of the panel test (Barbieri et al., 2020). One of these documents reports on the rules and practices to be applied during the selection, training, and monitoring of VOO tasters, panel leaders, and trainers that are fundamental to enhance the panel’s proficiency in recognizing, identifying, and quantifying sensory attributes (IOC/T.20/Doc. no.14/Rev.6, 2020). People who intend to become tasters of VOO are initially selected through the application of tests to verify their sensitivity to odors and tastes and discriminatory skills (e.g. determination of the detection threshold of panellist candidates and application of the intensity rating method); subsequently, during the training phase, tasters needs to become familiar with specific sensory methodology and sensory attributes (practical trials for recognizing by smell and taste the characteristic negative and positive attributes of VOO and their intensities; use the official profile sheet for classification of the various categories of VOO; tasting and becoming familiar with sensory characteristics of national/international olive varieties). Once the panel has been set up, its maintenance is performed through continuous training and by the application of quality control procedures, and verification of the sensory acuity of tasters and panel performance. Moreover, each year, all panels must undergo a proficiency test in which several reference samples must be assessed to verify the reliability of the results from different panels and to harmonize their perception criteria.

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

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

Two classes of materials are recognized by the ISO, namely certified RMs (CRMs) and RMs. CRMs must be accompanied by a certificate issued by a certifying body that attests the accurate procedure for obtaining the proper values that are expressed (traceability) with an uncertainty at a stated level of confidence. RMs are materials or substances whose property values are sufficiently homogeneous, stable, and well established for its intended use in a measurement process (ISO, 1992; ISO, 2000). Taking into account these required properties, some of the key issues that need to be considered in the preparation of RMs are the appropriateness of selected materials, homogeneity in testing, preparation (including packaging), and stability during distribution and storage (EA, 2003; Karambelkar, 2018).

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

Despite the extensive literature on the application of sensory analysis for VOO quality control, only a few studies concerning the use and assessment of RMs for VOO sensory analysis have been carried out. Some researchers have recently proposed the use of fingerprinting chromatographic signal as alternative technique to sensory analysis to monitor the stability RMs for VOO over time (Valverde-Som et al., 2018a; Valverde-Som et al., 2018b) and to assess their homogeneity (Ortega-Gavilán et al., 2020) by evaluating the similarity between fingerprint signals on the volatile fraction of samples. In a newly published study, carried out within the framework of the OLEUM project (Horizon 2020, Grant Agreement No. 635690), a strategy for designing RMs as supporting tools for panel training was presented and the optimization process to obtain the best formulation for rancid and winey-vinegary defects has been illustrated (Aparicio-Ruiz et al., 2020). Representativeness, aroma persistence, and simplicity in composition were the main criteria applied to obtain a formulation that, with an open-source composition, should offer several advantages including reproducible manufacturing of RMs and the possibility of in-house preparation or purchase of RM that are already prepared.

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

2. Materials and Methods

2.1 RMs for VOO and sensory panels

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

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

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

Kit for sensory evaluation of RMs
• 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 1.7[1] (code IT_35)
• Actual sample of VOO with an intensity of rancid defect of 2.3[1] (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

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

2.2 Technical protocol for sensory evaluation of RMs

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

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

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

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

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

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

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

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

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

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

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

3. Results and discussion

3.1 Evaluation of representativeness of RMs

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

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: [1]comment 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%.

3.2 Determination of detection threshold

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

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

3.3 Evaluation of long term stability.

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

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, $p < 0.05$).

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

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

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 %.

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

4. Conclusions

The proposed RMs were judged as representative of real samples of defected VOOs and exhibited suitable long-term stability. Furthermore, determination of the detection threshold of panels was effective in establishing the minimum perception limit, but also to certify different intensities of the proposed dilutions of RMs. In this way, it is possible to expand the range of activities in which these RMs can be applied for the selection, training, and monitoring of VOO tasters, panel leaders, and trainers. The possibility to prepare and use the indentical RMs in each sensory laboratory, strictly following an detailed open access protocol, could increase the homogeneity of results from panels. On the other hand, for labs that prefer to purchase RMs it is desirable that the present paper and the results of inter-laboratory tests (validation) which are still in progress, involving sensory panels from different countries, will promote their production and availability on the market. RMs are definitely be of interest for a large number of official and professional sensory panels for VOO evaluation worldwide (as well as associations that organize sensory courses to select and train new assessors). The introduction of new RMs does not have the aim of replacing those already in use, but rather to offer an alternative to single and "pure" defects, with training purposes.

In addition, future studies should be addressed to the development of RMs for other sensory defects, or to the application of other formulation methods such as fermentative and biotechnological production.

Author statement

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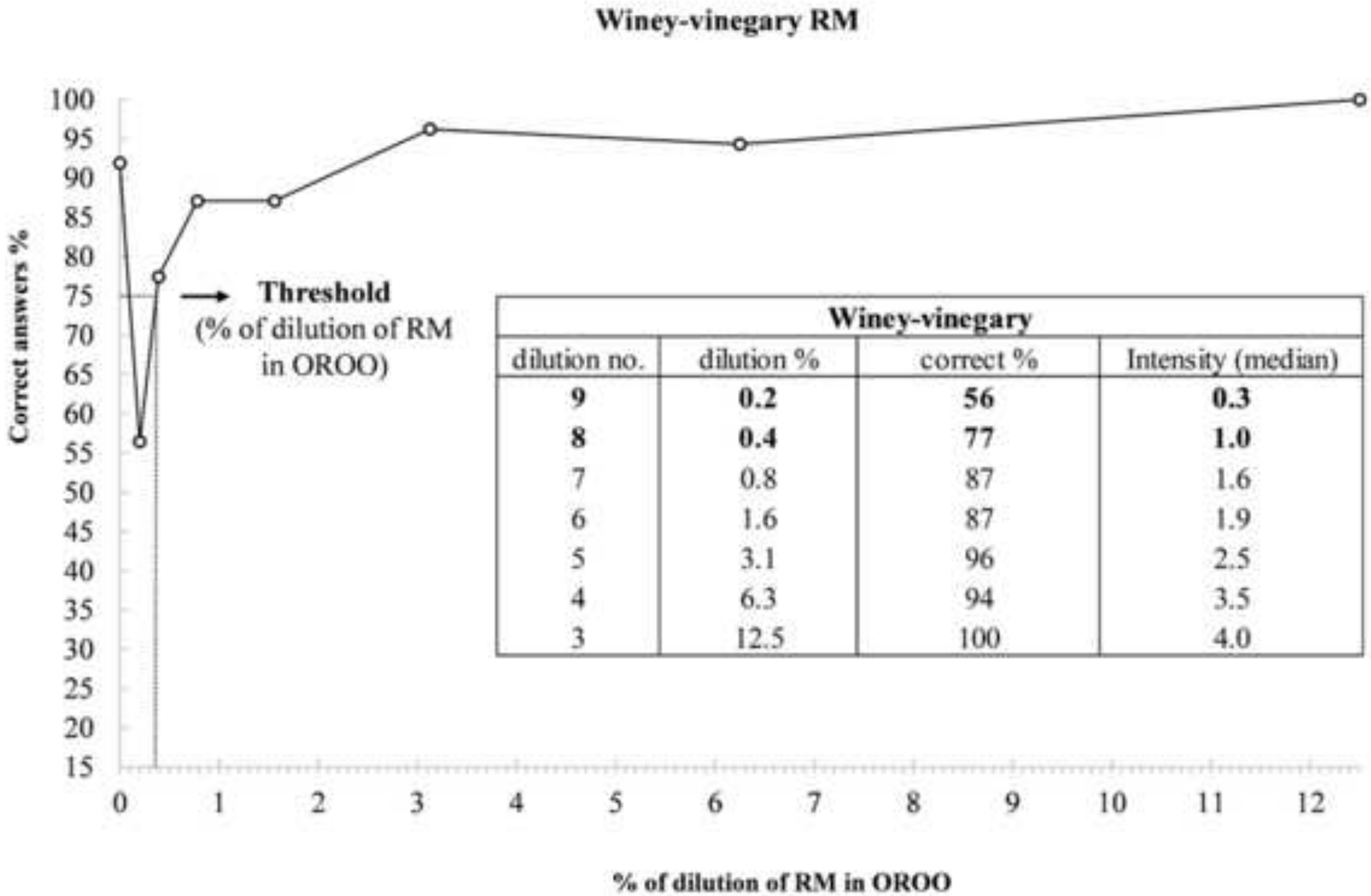
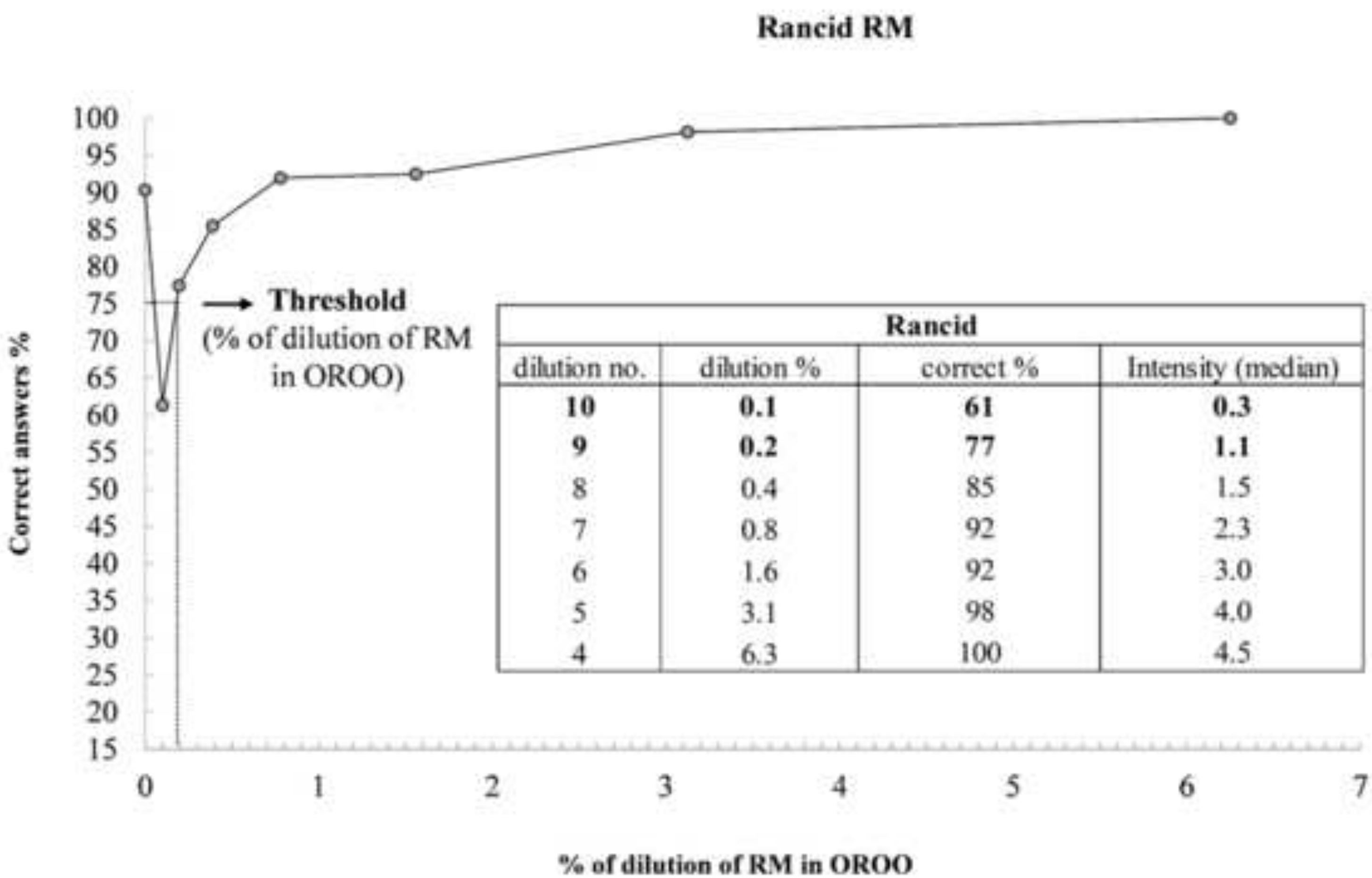


Winey-vinegary			
dilution no.	dilution %	correct %	Intensity (median)
9	**0.2**	**56**	**0.3**
8	**0.4**	**77**	**1.0**
7	0.8	87	1.6
6	1.6	87	1.9
5	3.1	96	2.5
4	6.3	94	3.5
3	12.5	100	4.0



Rancid			
dilution no.	dilution %	correct %	Intensity (median)
10	**0.1**	**61**	**0.3**
9	**0.2**	**77**	**1.1**
8	0.4	85	1.5
7	0.8	92	2.3
6	1.6	92	3.0
5	3.1	98	4.0
4	6.3	100	4.5

Tables

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

Kit for sensory evaluation of RMs
• 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 1.7[1] (code IT_35)
• Actual sample of VOO with an intensity of rancid defect of 2.3[1] (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

[1]Mean 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: [1]comment 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

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

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

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