This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 635690. The views and opinions expressed in this document are the sole responsibility of the author and do not necessarily reflect the views of the European Commission.

DELIVERABLE D3.9

Title: Report on the suitability of Reference Materials in the training and monitoring of panellists according to the "IOC Guide for the selection, training and monitoring of skilled virgin olive oil tasters"

Date: 15/01/2020

LEAD BENEFICIARY: UNIBO

OTHER BENEFICIARIES: CSIC, EUROFINS, IPTPO, ITERG, UZZK, ZRS.

Project funded by the European Commission within the Horizon 2020 Programme (2014- 2020)		
Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (Including the Commission Services)	
RE	Restricted to a group specified by the consortium (Including the Commission Services)	
CO	Confidential, only for members of the consortium (Including the Commission Services)	

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.

Table of contents

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.

1. Executive summary

The Panel test is both a qualitative and quantitative method, since its application results in the classification of samples based on the median of the predominant defect and the presence or not of the fruity attribute. For its use, it is necessary that tasters must be correctly trained and supervised for correct classification of samples and for correct recognition of the intensities of perceived attributes.

In this context, OLEUM project is engaged in reinforcing the methodology for the sensory evaluation improving its reproducibility by the adoption of supporting tools (reproducible sensory RMs) for training the panels and undergoing quality control of the panelists, through the design of a global procedure named Quantitative Panel Test.

The introduction of RMs obtained by mixing pure molecules in specific concentrations, in future reachable on the market, will allow to overcome some limits of those obtained from natural matrices and will offer several advantages including the reproducibility over time, and/or the possibility of purchase, which would mean unlimited availability.

Two new artificial RMs (for the aroma of winey-vinegary and rancid defects) have been formulated (for details on formulation, see D.3.6) ad hoc for resembling these sensory defects using specific mixtures of volatile molecules at specific concentrations relevant for the sensory attribute to be emulated and based on their odorous threshold (selection of volatile compounds obtained after a deep study of sample profiles characterized by the presence of specific sensory descriptors).

A protocol for the RMs practical application has been defined and shared with the six sensory panels involved in the project; it includes three different parts: the evaluation of their representativeness (with respect to real samples presenting the sensory defect); the determination of panels' detection threshold for each one and their shelf-life evaluation over time.

The stock solutions of each reference material and odorless refined olive oil needed for the detection threshold evaluation were shipped to sensory panels and all sensory results were collected for their processing.

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.

2. Materials and Methods

2.1 Analyzed samples

Stock solutions of each RMs (winey-vinegary, coded as AV8 and rancid, coded as R7), formulated as reported in D.3.6, were prepared by UNIBO panel that is responsible of the coordination of all sensory activities.

Each stock solution was divided into aliquots and sent to the other panels. A storage at 10-12°C in darkness conditions and the reconditioning at room temperature 6-8 hours before analysis were recommended.

Considering this activity as preliminary step (revisions in the formulation of reference materials could be proposed by panel leaders to UNIBO/CSIC for possible improvements), only a small quantity of the RM stock solutions was prepared and shipped to the other panels (EUROFINS, IPTPO, ITERG, ZRS, UZZK).

A technical protocol for the sensory evaluation of RMs (only by smell) including details for the preparation of the RMs sensory session was defined and distributed with all the panels.

Each sensory panel is provided with:

- RM of winey-vinegary stock solution (AV8) in a plastic bottle (50 ml);
- RM of rancid stock solution (R7) in a plastic bottle (50 ml);
- Odorless refined olive oil (OROO) needed for their dilutions in steel tins (2 x 300 ml);
- Empty plastic bottles for dilutions (24 x 50 ml);
- RM evaluation sheet to be used by both panel leaders and assessors.

2.2 Technical protocol for sensory evaluation of RMs

2.1. Evaluation of representativeness

Each assessor is required to evaluate the representativeness of the stock solution of each RMs (AV8 and R7) and to provide their intensity comparing them with real samples from the first selection of the OLEUM sampling 2018 (ITERG_T3.1_35 for

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.

winey-vinegary defect and IPTPO_T3.1_45 for rancid defect), using the first part of the RM evaluation sheet (please, see **annexes 1 and 2**).

Each panel leader is asked to collect their own sensory data (panel leader + 8 assessors) using the dedicated excel file ("excel file for RMs sensory data", sheet named "first part").

2.2 Determination of the detection of the panels' threshold

For each stock solution of RMs (AV8 and R7) successive dilutions using the same methodology applied by panels for the detection threshold of the group of candidates for characteristic attributes (COI/T.20/Doc. No 14/Rev. 5, 2018) were prepared.

These dilutions were used to determine the detection threshold of each panel (panel leader + 8 assessors) and to evaluate their intensity of perceptions (with a linear scale).

The determination of the detection threshold was carried out with paired difference tests for each of the two RMs (AV8 and R7) and OROO, where the tasters are not called to recognize the attribute but only to state if they detect a stimulus and quantifying its intensity.

Each panel leader prepared a series of samples for each of stock solution (AV8 and R7) in descending concentrations (dilutions 1:1 v:v) by making successive dilutions in OROO (please, see **note 1**). The series were considered complete when no difference could be detected between two successive dilutions and the OROO. The panel leader shall then choose the seven dilutions prior to these last two (please, see **note 2**).

Paired difference tests by smelling shall be carried out to determine the detection threshold of panel leader and each assessor and thus to establish the detection threshold of the panel.

Each assessor (also the panel leader) is presented with up to a total of 8 pairs of samples, randomly presented and in successively independent tests (the pairs comprise one of each of the seven samples chosen and OROO as blank medium, plus one pair of OROO).

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.

In each test, the candidates shall be asked if the two samples (using different codes for each dilution) are identical or different and its intensity using the second part of the RM evaluation sheet (please, see **annex 1 and 2**).

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

Moreover, the intensity of each of the 7 dilutions (chosen by the panel leader) of each stock solution (AV8 and R7) must be provided by the panellists.

Considering the limited amount of RM stock solutions available, only one booth has been set up and the test was run by tasters one by one. The use of device for heating samples (28 ± 2°C), covers for glasses and a break of some minutes between the tests, were recommended.

Each panel leader was required to collect their own sensory data (panel leader + 8 assessors) using the dedicated excel file ("excel file for RMs sensory data", sheet named "second part").

Note 1. Procedure for preparing dilutions:

Stock solution (AV8 and R7) (50 ml)

Dilution 1: withdraw 20 g from stock solution (AV8 and R7) and add 20 g of OROO

Dilution 2: withdraw 20 g from dilution 1 and add 20 g of OROO

Dilution 3: withdraw 20 g from dilution 2 and add 20 g of OROO

Dilution 4: withdraw 20 g from dilution 3 and add 20 g of OROO

Dilution 5: withdraw 20 g from dilution 4 and add 20 g of OROO

Dilution 6: withdraw 20 g from dilution 5 and add 20 g of OROO

Dilution 7: withdraw 20 g from dilution 6 and add 20 g of OROO

Dilution 8: withdraw 20 g from dilution 7 and add 20 g of OROO

Dilution 9: withdraw 20 g from dilution 8 and add 20 g of OROO

Dilution 10: withdraw 20 g from dilution 9 and add 20 g of OROO

and so on until no difference can be detected between two successive dilutions and the OROO by the panel leader.

Note 2. Example for choosing the "7 dilutions" by the panel leader:

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.

The panel leader detects no difference between dilution n.11 and OROO and between dilution n.12 and OROO; he or she must choose dilutions 10-9-8-7-6-5-4 to be proposed to assessors by paired difference test and for the intensity evaluation.

2.3 Shelf life evaluation

Shelf life evaluation was also necessary to provide important information to detect any modifications during the storage period and estimate the best before dates of each RM. For this reason, the sensory evaluation (only evaluation of the intensities by smell) of 7 dilutions of each RM (previously chosen by panel leader) and its stock solution (AV8 and R7) was also scheduled after 3, 6, 9 and 12 months, using the third part of the RM evaluation sheet (please, see **annexes 1 and 2**).
Therefore, it was necessary to store all RMs (stock solutions and its dilutions) in such a manner as to protect the samples from light and heat, avoiding contamination from extraneous material. A temperature of around 10-12°C was recommended.
Each panel leader is asked to collect their own sensory data (panel leader + 8 assessors) using the dedicated excel file ("excel file for RMs sensory data", sheet named "third part").

3. Results and discussion

3.1 Evaluation of representativeness

As regards the assessment of the representativeness of the two reference materials (first part of the technical protocol), the majority of tasters believed that both the reference materials tested were representative of the related defects. Specifically, in the case of winey-vinegary, 97% of assessors considered the RM to be useful and assigned an intensity value of 8.0 (mean of the medians) but in some cases it was considered too high (**Table 1**). For the rancid, 82% of assessors considered the RM to be useful and assigned an intensity value of 8.0 (mean of the medians) but some

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.

tasters specified that the RM was different from the real defect due to the predominance of other secondary odours such as fried and/or bedbug (**Table 1**).

RM	% useful	Intensity	Comments
Winey-vinegary	97	8.0	too intense*
Rancid	82	8.0	presence of odours other than rancid (e.g. bedbug, fried)**

**these comments were provided by 2/6 panels.*

***these comments were provided by 1/6 panels.*

Table 1. Results from the first part of the protocol on the practical application of the new RMs. These data are related to the stock solution of each RM: Winey-vinegary (AV8) and Rancid (R7).

3.2 Determination of the detection of the panels' threshold

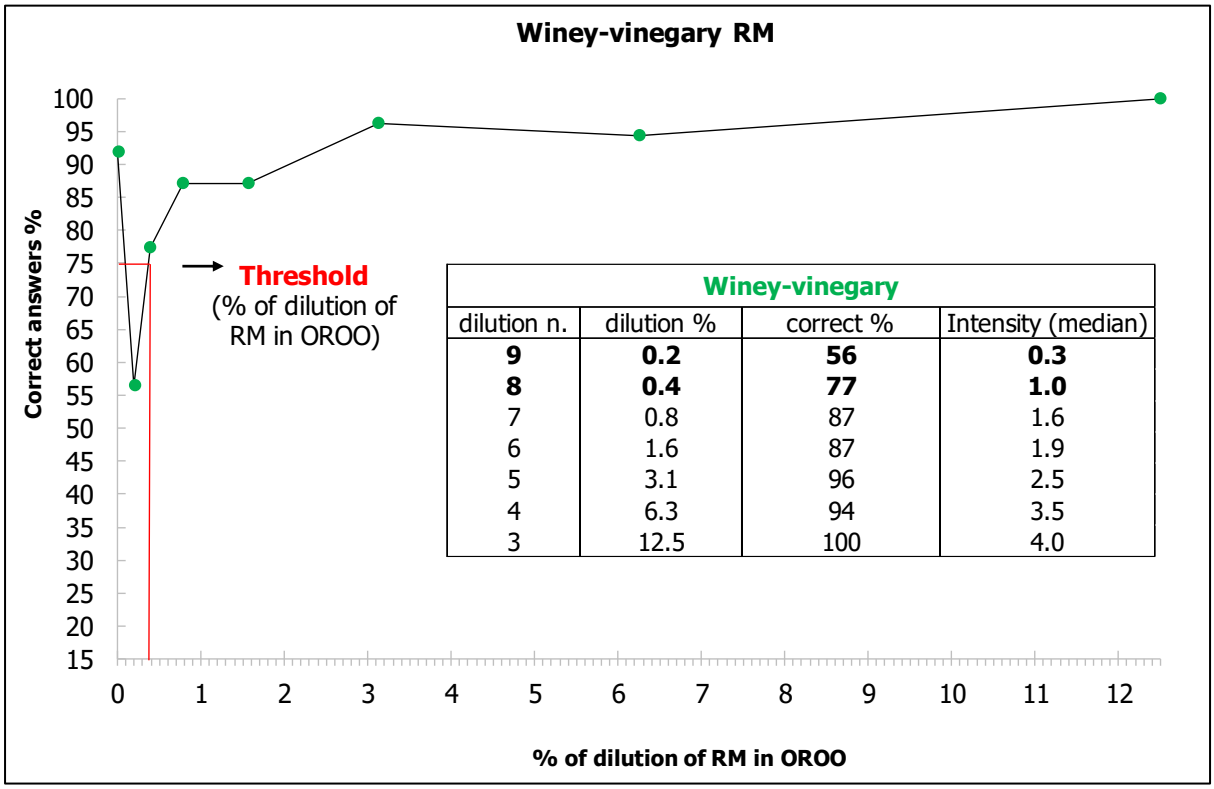
The second part of the protocol provided for the determination of the average perception threshold of the six OLEUM panels. The methodology described in the protocol provided to each panel was the same as reported in the document COI/T.20 doc.n.14 "Guide for the selection, training and control of qualified virgin olive oil tasters". To determine the average threshold of each panel and subsequently that of the six panels, each panel leader had to prepare a series of dilutions with decreasing concentrations for successive dilutions 1:1 for each mother solution of the two sensory defects, operating as previously described (see section 2.2).

After the comparison tests carried out on a total of 8 pairs per taster (7 dilutions and one control), each panel leader sent their results to UNIBO who processed data, noting the correct answers for each concentration and expressing them in percentage; the

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.

detection threshold corresponds, in fact, to the corresponding concentration (interpolation) to 75% of the correct answers.

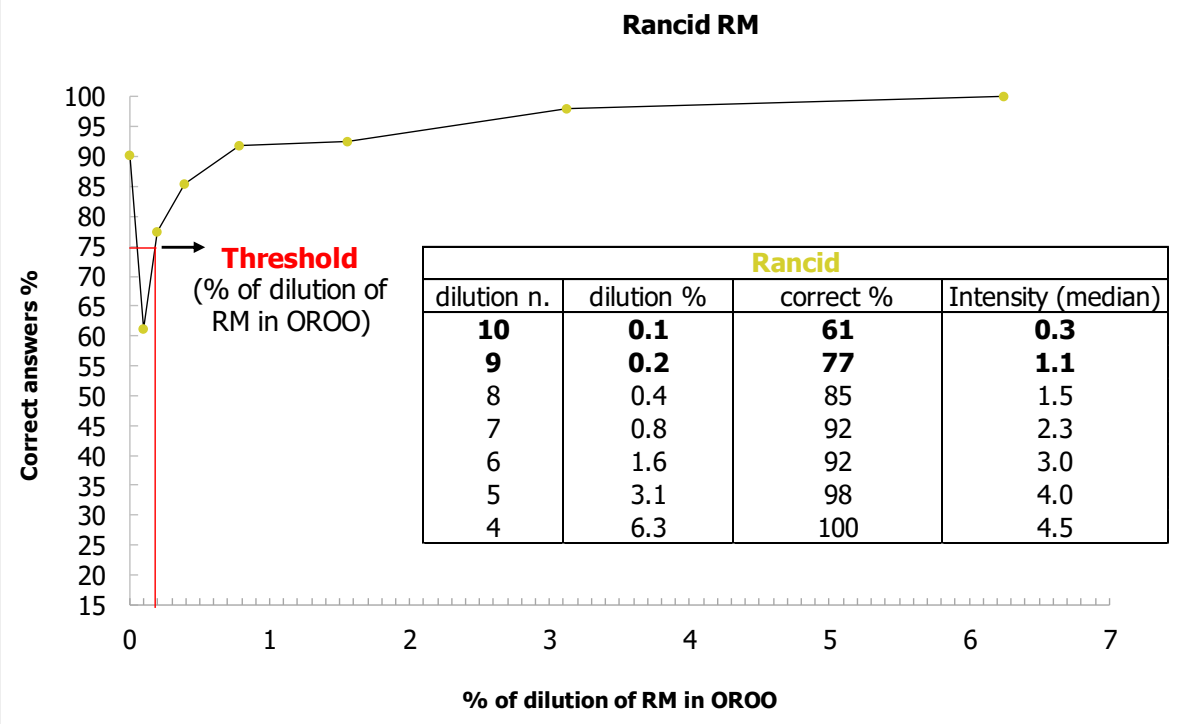
In **Figures 1** and **2**, the values of the detection thresholds of the six OLEUM panels (mean values) for the RMs of winey-vinegary and rancid, are shown.



Winey-vinegary			
dilution n.	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

Figure 1. Results from the second part of the protocol on the practical application of the new RMs. These data are related to the dilutions prepared, to the number of correct answers provided for each comparison carried out and to the perceived intensity for the 7 dilutions of the stock solution of Winey-vinegary RM.

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.



Rancid			
dilution n.	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

Figure 2. Results from the second part of the protocol on the practical application of the new RMs. These data are related to the dilutions prepared, to the number of correct answers provided for each comparison carried out and to the perceived intensity for the 7 dilutions of the stock solution of Rancid RM.

Observing these data, it emerges that the detection threshold of the panel for the winey-vinegary defect lies between dilutions 8 and 9 which correspond to a perceived intensity of between 0.3 and 1 and a concentration between 0.2 and 0.4%; in the case of rancid defect, the detection threshold of the panel lies between dilutions 9 and 10, which correspond to a perceived intensity of between 0.3 and 1.1 and a concentration between 0.1 and 0.2%.

3.3 Shelf life evaluation

Finally, in the third part, tasters from six panels were asked to re-evaluate the stock solutions and the 7 dilutions of each RM after 3, 6, 9 and 12 months from their preparation (at now data are available for 0, 3 and 6 months being the evaluation

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.

related to 9 months and 12 months scheduled for M36 and M39, respectively). Results of the comparison between time 0, time 1 (3 months) and time 2 (6 months) are shown in **Table 2** (great median of all values provided by all assessors from each panel) and **Table 3** (mean values).

Winey-vinegary RM shelf-life	Stock solution	Dil.3	Dil.4	Dil.5	Dil.6	Dil.7	Dil.8	Dil.9
GM_time 0	8.0	4.5	3.9	2.5	1.9	1.6	1.0	0.3
GM_time 1	7.5	4.5	3.5	2.1	2.4	2.0	1.2	0.8
GM_time 2	8.1	4.1	3.5	2.9	2.5	1.6	1.4	0.5
Rancid RM shelf-life	**Stock solution**	**Dil.4**	**Dil.5**	**Dil.6**	**Dil.7**	**Dil.8**	**Dil.9**	**Dil.10**
GM_time 0	8.0	4.5	4.0	3.0	2.3	1.5	1.1	0.3
GM_time 1	8.0	4.8	5.0	3.7	3.5	2.2	1.7	1.0
GM_time 2	8.0	6.0	5.0	4.5	4.0	3.0	2.5	2.2

Table 2. Results from the third part of the protocol on the practical application of the new RMs. These data are related to the intensity (great median, GM) of the stock solution and of the 7 dilutions of RM for winey-vinegary defect at time 0, after 3 and after 6 months of storage.

The XLSTAT 7.5.2 software (Addinsoft, France) was used for processing the data by the application of analysis of variance (ANOVA) followed by Fishers, LSD post-hoc test ($p < 0.05$) in order to highlight possible significant differences between the samples. Results showed that there were no significant differences for the winey-vinegary RM; only in the dilution n.6 the intensity significantly increased when passing from 0 to 3 months but comparing time 0 with time 2 no significant differences were found. In the case of rancid RM, there is a clear tendency of the intensity to increase when passing from 0 to 3 or 6 months (significant sometimes between 0 and 3 months and sometimes between 0 and 6 months); this increase in the perceived intensity of defect occurred probably due to the oxidation of the refined olive oil (OROO) used for preparing dilutions that inevitably occurs over time.

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.

Winey-vinegary RM shelf-life	Stock solution	Dil.3	Dil.4	Dil.5	Dil.6	Dil.7	Dil.8	Dil.9
Mean_time 0	7.7^{a}	4.6^{a}	3.9^{a}	2.8^{a}	2.0^{b}	1.7^{a}	1.3^{a}	0.8^{a}
Mean_time 1	7.4^{a}	4.7^{a}	3.8^{a}	2.5^{a}	2.5^{a}	2.1^{a}	1.4^{a}	0.9^{a}
Mean_time 2	7.4^{a}	4.4^{a}	3.4^{a}	2.8^{a}	2.4^{ab}	1.7^{a}	1.4^{a}	0.9^{a}
Rancid RM shelf-life	**Stock solution**	**Dil.4**	**Dil.5**	**Dil.6**	**Dil.7**	**Dil.8**	**Dil.9**	**Dil.10**
Mean_time 0	7.9^{b}	4.4^{b}	4.1^{b}	3.0^{b}	2.6^{b}	1.7^{b}	1.4^{b}	0.6^{b}
Mean_time 1	8.2^{ab}	4.9^{b}	4.8^{ab}	4.0^{a}	3.5^{a}	2.3^{a}	1.8^{a}	1.2^{a}
Mean_time 2	8.5^{a}	5.9^{a}	4.9^{a}	3.6^{ab}	3.0^{b}	2.2^{a}	1.6^{ab}	1.0^{a}

Table 3. Results from the third part of the protocol on the practical application of the new RMs. These data are related to the intensity (mean values) of the stock solution and of the 7 dilutions of winey-vinegary defect at time 0, after 3 and after 6 months of storage. Different letters in the same column indicate significant differences (Fisher LSD, $p<0.05$).

4. Conclusions

The practical application of the two new materials (winey-vinegary and rancid) carried out by the 6 panels involved in the OLEUM activities highlighted their efficacy in representing the corresponding defects. The possible introduction of new artificial RMs to support the training of tasters and increase the proficiency of the sensory panels could improve the effectiveness of the method (selection, training and monitoring of skilled virgin olive oil tasters) by overcoming some of the limitations typical of those obtained from natural matrices and offering advantages as the reproducibility over time and/or the possibility of purchase which would therefore mean their unlimited availability.

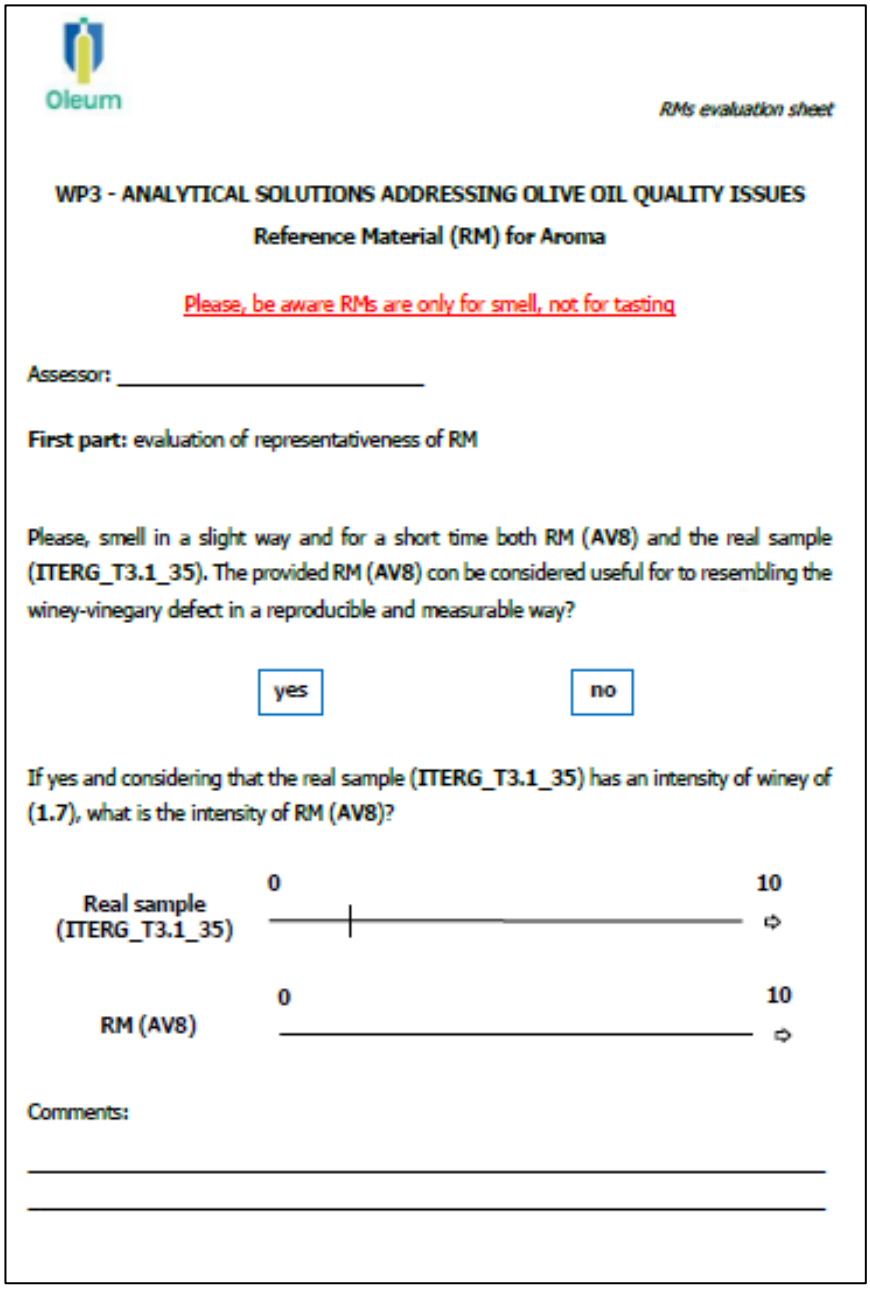
This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.

5. Bibliography

COI/T.20/Doc. No 14/Rev. 5, 2018. Guide for the selection, training and quality control of virgin olive oil tasters-qualifications of tasters, panel leaders and trainers. Internacional Olive Council (IOC).

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.

Annex 1. *RMs evaluation sheet for winey-vinegary (A1 a-c) and rancid (A2 a-c).*



Oleum

RMs evaluation sheet

WP3 - ANALYTICAL SOLUTIONS ADDRESSING OLIVE OIL QUALITY ISSUES

Reference Material (RM) for Aroma

Please, be aware RMs are only for smell, not for tasting

Assessor: _____

First part: evaluation of representativeness of RM

Please, smell in a slight way and for a short time both RM (**AV8**) and the real sample (**ITERG_T3.1_35**). The provided RM (**AV8**) con be considered useful for to resembling the winey-vinegary defect in a reproducible and measurable way?

yes no

If yes and considering that the real sample (**ITERG_T3.1_35**) has an intensity of winey of (**1.7**), what is the intensity of RM (**AV8**)?

Real sample (ITERG_T3.1_35) 0 10

RM (AV8) 0 10

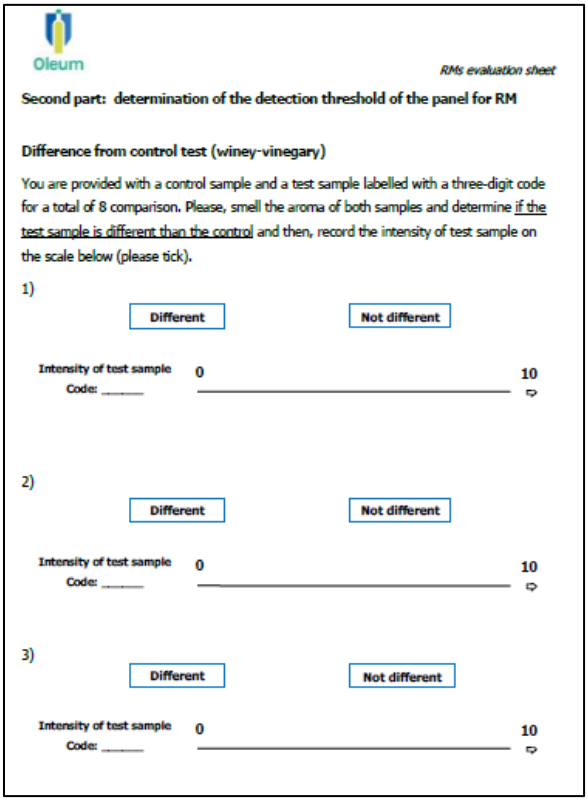
Comments:

_____

_____

A.1a RMs evaluation sheet for winey-vinegary (first part).

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.



Oleum

RMs evaluation sheet

Second part: determination of the detection threshold of the panel for RM

Difference from control test (winey-vinegary)

You are provided with a control sample and a test sample labelled with a three-digit code for a total of 8 comparison. Please, smell the aroma of both samples and determine if the test sample is different than the control and then, record the intensity of test sample on the scale below (please tick).

1)

Different | Not different

Intensity of test sample Code: _____ 0 ——— 10

2)

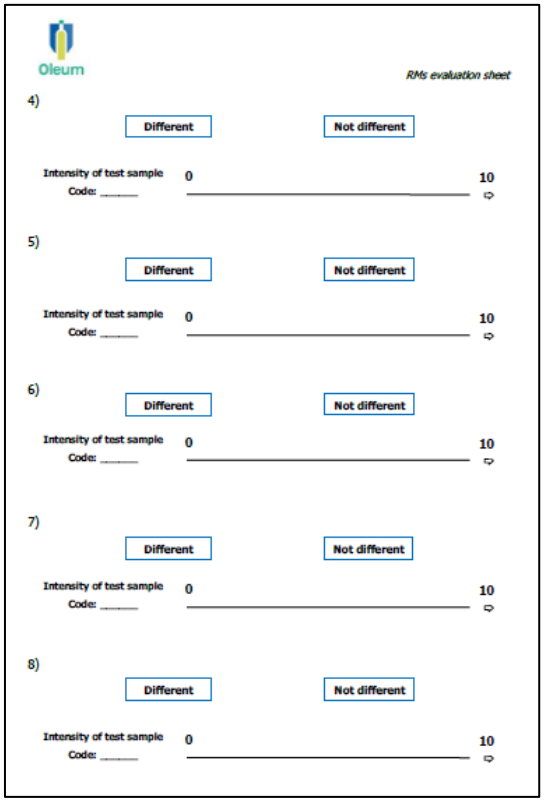
Different | Not different

Intensity of test sample Code: _____ 0 ——— 10

3)

Different | Not different

Intensity of test sample Code: _____ 0 ——— 10



Oleum

RMs evaluation sheet

4)

Different | Not different

Intensity of test sample Code: _____ 0 ——— 10

5)

Different | Not different

Intensity of test sample Code: _____ 0 ——— 10

6)

Different | Not different

Intensity of test sample Code: _____ 0 ——— 10

7)

Different | Not different

Intensity of test sample Code: _____ 0 ——— 10

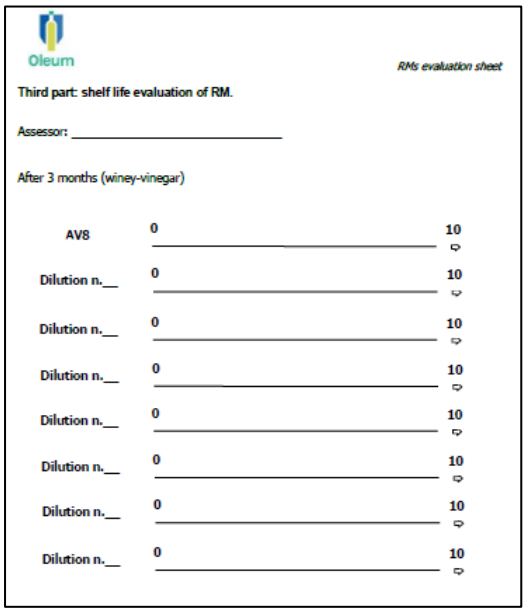
8)

Different | Not different

Intensity of test sample Code: _____ 0 ——— 10

A.1b RMs evaluation sheet for winey-vinegary (second part).

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.


Oleum

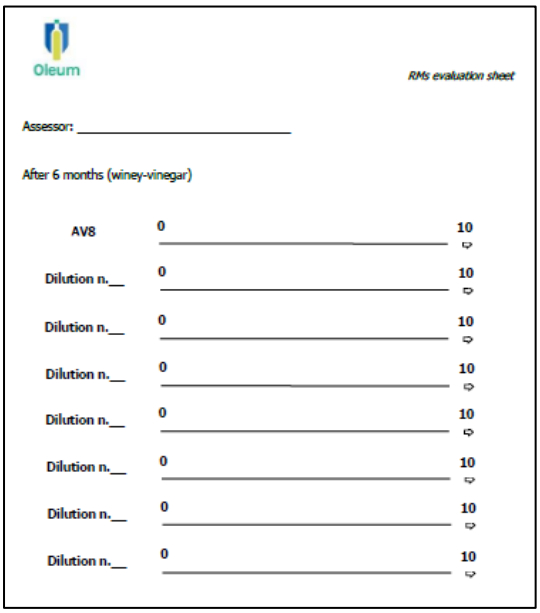
RMs evaluation sheet

Third part: shelf life evaluation of RM.

Assessor: _____

After 3 months (winey-vinegar)

AV8	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10

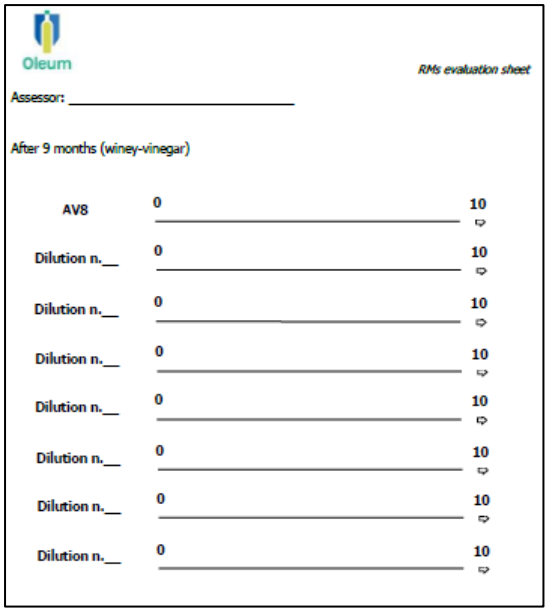

Oleum

RMs evaluation sheet

Assessor: _____

After 6 months (winey-vinegar)

AV8	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10

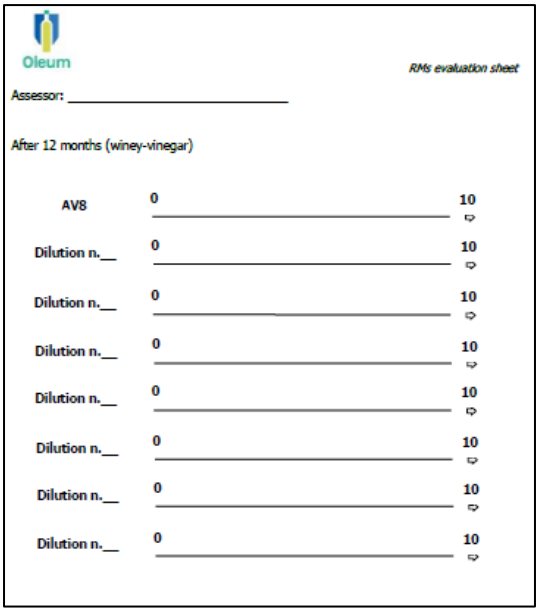

Oleum

RMs evaluation sheet

Assessor: _____

After 9 months (winey-vinegar)

AV8	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10


Oleum

RMs evaluation sheet

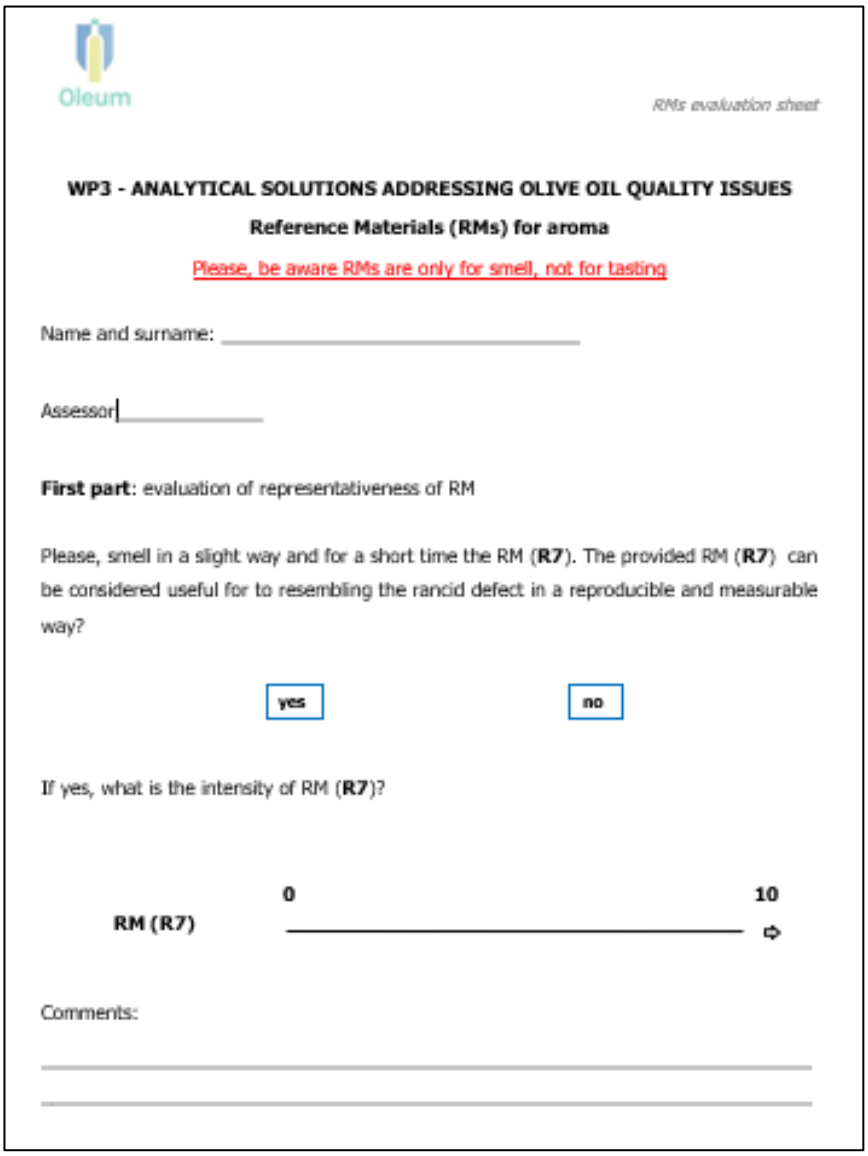
Assessor: _____

After 12 months (winey-vinegar)

AV8	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10
Dilution n.__	0 _____	10

A.1c RMs evaluation sheet for winey-vinegary (third part).

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.



Oleum

RMs evaluation sheet

WP3 - ANALYTICAL SOLUTIONS ADDRESSING OLIVE OIL QUALITY ISSUES

Reference Materials (RMs) for aroma

Please, be aware RMs are only for smell, not for tasting

Name and surname: _____

Assessor_____

First part: evaluation of representativeness of RM

Please, smell in a slight way and for a short time the RM (**R7**). The provided RM (**R7**) can be considered useful for to resembling the rancid defect in a reproducible and measurable way?

yes no

If yes, what is the intensity of RM (**R7**)?

	0	10
RM (R7)	———	⇨

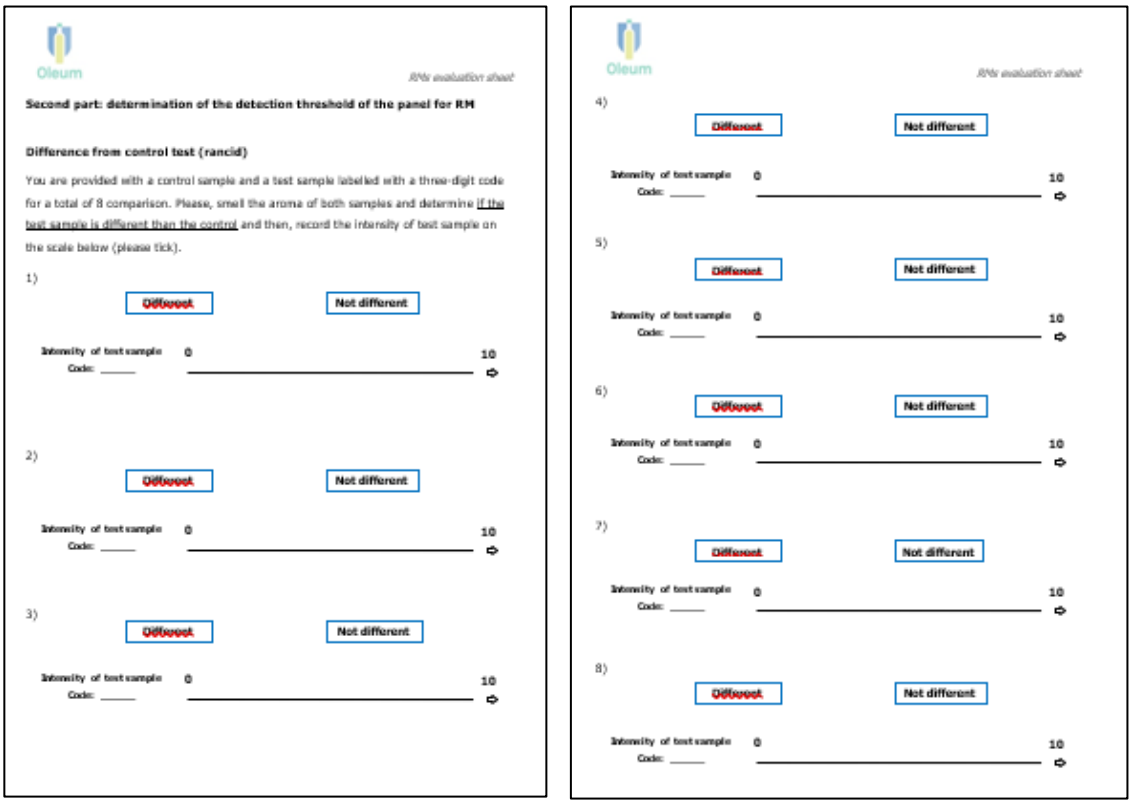
Comments:

_____

_____

A.2a RMs evaluation sheet for rancid (first part).

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.



Oleum

RMs evaluation sheet

Second part: determination of the detection threshold of the panel for RM

Difference from control test (rancid)

You are provided with a control sample and a test sample labeled with a three-digit code for a total of 8 comparison. Please, smell the aroma of both samples and determine if the test sample is different than the control and then, record the intensity of test sample on the scale below (please tick).

1) Different / Not different

Intensity of test sample Code: _____ 0 ——— 10

2) Different / Not different

Intensity of test sample Code: _____ 0 ——— 10

3) Different / Not different

Intensity of test sample Code: _____ 0 ——— 10

4) Different / Not different

Intensity of test sample Code: _____ 0 ——— 10

5) Different / Not different

Intensity of test sample Code: _____ 0 ——— 10

6) Different / Not different

Intensity of test sample Code: _____ 0 ——— 10

7) Different / Not different

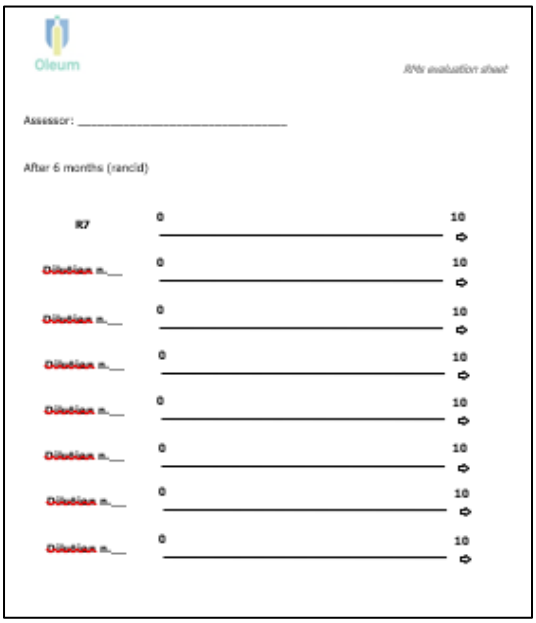
Intensity of test sample Code: _____ 0 ——— 10

8) Different / Not different

Intensity of test sample Code: _____ 0 ——— 10

A.2b RMs evaluation sheet for rancid (second part).

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.



A.2c RMs evaluation sheet for rancid (third part).

This project has received funding from the European Union's
Horizon 2020 research and innovation programme under grant agreement No 635690.