COLLABORATIVE PEER VALIDATION OF A HARMONIZED SPME-GC-MS METHOD FOR ANALYSIS OF SELECTED VOLATILE COMPOUNDS IN VIRGIN OLIVE OILS

Ramón APARICIO-RUIZ[a], Clemente ORTIZ ROMERO[a], Enrico CASADEI[b], Diego L. GARCÍA-GONZÁLEZ[a], Maurizio SERVILI[c], Roberto SELVAGGINI[c], Florence LACOSTE[d], Julien ESCOBESSA[d], Stefania VICHI[e], Beatriz QUINTANILLA-CASAS[e], Pierre-Alain GOLAY[f], Paolo LUCCI[g], Erica MORET[g], Enrico VALLI[b]✉, Alessandra BENDINI[b], Tullia GALLINA TOSCHI[b]

[a]Instituto de la Grasa (CSIC), Sevilla, Spain; [b]Department of Agricultural and Food Sciences, Alma Mater Studiorum – Università di Bologna, Bologna, Italy; [c]Department of Agricultural, Food and Environmental Sciences, Università degli Studi di Perugia, Perugia, Italy; [d]ITERG (Institut des Corps Gras), Canejan, France; [e]Department de Nutrició, Ciències de L'Alimentación Gastronomia/INSA-UB, Universitat de Barcelona, Santa Coloma de Gramenet, Spain; [f]Nestlé Research Center, Lausanne, Switzerland; [g]Department of Agri-Food, Animal and Environmental Science Università degli Studi di Udine, Udine, Italy;

✉Corresponding author: Dr. Enrico Valli, PhD. E-Mail: enrico.valli4@unibo.it.

INTRODUCTION

The evaluation of the sensory attributes in virgin olive oil (VOO) is carried out according to the standard method of panel test [1, 2]. Recently, it has been proposed as SPME-GC-FID method as the most adequate instrumental method to determine volatiles compounds in VOO to support the sensory panels test [3]. This method developed in the European funded project OLEUM, in which all the variables have been defined and harmonized, has been tested in three laboratories to perform an inter-laboratory validation of the quantification of the most relevant VOCs that are responsible for VOO sensory attributes. The validation study was carried out for each one of the selected molecules in order to have an individual information for each analyte. With the same objective, in the present work, five laboratories, all being active partners in the OLEUM project, carried out an inter-lab evaluation of the SPME-GC-MS joint protocol. The validation was carried out by each laboratory following the same analytical conditions and on the same samples, in order to make the results obtained by each laboratory comparable in a harmonized procedure and methodology, as previously done with FID [3].

RESULTS

The SPME-GC-MS method for determination of volatile compounds in virgin olive oil responsible for positive attributes (e.g. fruity) and the main sensory defects had a linearity (R^2 > 0.94) and repeatability (mean relative standard deviation, RSD% = 7.60%) satisfactory (Table I). Reproducibility results were uneven depending on the compound. The lowest RSD% values were found for *(Z)*-3-hexenyl acetate (19.19%), 1-hexanol (13.26%), and acetic acid (17.47%). The limits of quantification were < 0.07 mg/kg for all compounds except for *(E)*-2-decenal and pentanoic acid. The study of different quantification methods revealed that the correction of the calibration curves using the internal standard led to a slightly worse repeatability, but better accuracy and reproducibility. This study was considered as a peer-study prior to a full validation process.

BIBLIOGRAPHY

[1] International Olive Council (IOC). Sensory analysis of olive oil method for the organoleptic assessment of virgin olive oil. IOOC/T.20/Doc. no. 3 (1987).

[2] European Commission Regulation. On the characteristics of olive oil and olive residue oil and on the relevant methods of analysis, and subsequent amendments. Official Journal of European Community, 11(L248), 1-102, 2568/91 (1991).

[3] E. Casadei, E. Valli, R. Aparicio-Ruiz, C. Ortiz-Romero, D.L. García-González, S. Vichi, B. Quintanilla-Casas, A. Tres, A. Bendini, T. Gallina Toschi, Peer inter-laboratory validation study of a harmo-

nized SPME-GC-FID method for the analysis of selected volatile compounds in virgin olive oils. Food Control 123, 107823 (2021).

Table I - Linearity expressed as R^2 (mean and standard deviation of the five labs) computed from the calibration curves used in the quantification methods 1 and 2 (QM1, QM2) for the 18 volatile compounds. Repeatability expressed as mean RSD%.

Volatile compounds	Linearity (R^2)		RSD% (Mean±SD)		
	QM1	QM2	QM1	QM2	QM3
Octane	0.996±0.003	0.966±0.038[a]	6.77±4.33[c]	7.95±4.11	6.47±4.91
Ethyl acetate	0.982±0.023[a]	0.906±0.078[a]	6.99±3.49	4.77±0.21	5.75±4.02
Ethanol	0.984±0.011[a]	0.953±0.047[a]	9.51±2.72	6.21±2.14	6.52±1.94
Ethyl propanoate	0.994±0.008	0.939±0.053[a]	15.27±15.87[c]	15.55±15.63	15.13±17.34
Hexanal	0.996±0.003	0.980±0.021	5.49±3.67	4.84±2.00	4.53±1.94
3-Methyl-1-butanol	0.996±0.002	0.941±0.068	5.09±1.80	5.63±2.58	2.88±2.44
(*E*)-2-Hexenal	0.990±0.009[b]	0.994±0.007[b]	4.15±1.74	2.99±0.40	2.21±1.30
(*Z*)-3-Hexenyl acetate	0.987±0.012[b]	0.992±0.006[b]	5.23±0.55[e]	4.86±0.84[d]	3.11±0.61[ef]
(*E*)-2-Heptenal	0.976±0.027[b]	0.997±0.001	5.38±0.76	4.75±4.23	3.31±3.61
6-Methyl-5-hepten-2-one	0.975±0.025[b]	0.997±0.001	5.05±1.17	5.82±0.89	4.40±0.07
1-Hexanol	0.993±0.006	0.992±0.005	3.89±1.46	4.12±0.72[f]	2.39±0.34[f]
Nonanal	0.976±0.024	0.990±0.007	11.84±7.33[c]	9.89±3.96	7.36±9.39
1-Octen-3-ol	0.983±0.019	0.993±0.005	6.98±1.59	5.40±0.98	5.84±3.03
(*E,E*)-2,4-Hexadienal	0.975±0.027	0.997±0.002	8.51±2.99	4.20±0.72	6.79±5.13
Acetic acid	0.993±0.005	0.989±0.011	7.87±0.47[d]	3.48±2.59[d]	5.48±3.09
Propanoic acid	0.983±0.028[b]	0.995±0.005	5.70±0.19[d]	2.35±1.56[d]	3.32±2.08
(*E*)-2-Decenal	0.942±0.057[b]	0.966±0.025[b]	17.23±5.08[e]	12.00±2.77	13.86±5.10[e]
Pentanoic acid	0.969±0.032[b]	0.993±0.008[b]	5.83±0.27[d]	3.17±0.58[d]	2.83±1.86

[a] Certain saturation at high concentrations in data provided by some of the involved labs.
[b] Certain lower sensitivity (lower slope) at low concentrations in data provided by some of the involved labs.
[c] One outlier has been removed (Grubbs test $p<0.05$).
[d] Significant difference ($p<0.05$) between QM1 and QM2.
[e] Significant difference ($p<0.05$) between QM1 and QM3.
[f] Significant difference ($p<0.05$) between QM2 and QM3.

This abstract is based on the published article R. Aparicio-Ruiz, C. Ortiz Romero, E. Casadei, D.L. García-González, M. Servili, R. Selvaggini, F. Lacoste, J. Escobessa, S. Vichi, B. Quintanilla-Casas, P.A. Golay, P. Lucci, E. Moret, E. Valli, A. Bendini, T. Gallina Toschi, Collaborative peer validation of a harmonized SPME-GC-MS method for analysis of selected volatile compounds in virgin olive oils. Food Control (2022), doi: https://doi.org/10.1016/j.foodcont.2021.108756.

INTERVALIDAZIONE DI UN METODO ARMONIZZATO SPME-GC-MS PER L'ANALISI DI COMPOSTI VOLATILI SELEZIONATI IN OLI DI OLIVA VERGINI

Ramón APARICIO-RUIZ[a], Clemente ORTIZ ROMERO[a], Enrico CASADEI[b], Diego L. GARCÍA-GONZÁLEZ[a], Maurizio SERVILI[b], Roberto SELVAGGINI[b], Florence LACOSTE[c], Julien ESCOBESSA[d], Stefania VICHI[d], Beatriz QUINTANILLA-CASAS[d], Pierre-Alain GOLAY[e], Paolo LUCCI[f], Erica MORET[f], Enrico VALLI[g]✉, Alessandra BENDINI[g], Tullia GALLINA TOSCHI[g]

[a]Instituto de la Grasa (CSIC), Sevilla, Spain; [b]Department of Agricultural, Food and Environmental Sciences, Università degli Studi di Perugia, Perugia, Italy; [c]ITERG (Institut des Corps Gras), Canejan, France; [d]Department de Nutrició, Ciències de L'Alimentació I Gastronomia/INSA-UB, Universitat de Barcelona, Santa Coloma de Gramenet, Spain; [e]Nestlé Research Center, Lausanne, Switzerland; [f]Department of Agri-Food, Animal and Environmental Science Università degli Studi di Udine, Udine, Italy; [g]Department of Agricultural and Food Sciences, Alma Mater Studiorum – Università di Bologna, Bologna, Italy.

✉Dr. Enrico Valli, PhD. E-Mail: enrico.valli4@unibo.it.

INTRODUZIONE

La valutazione degli attributi sensoriali degli oli di oliva vergini (VOO) è svolta in accordo al metodo ufficiale noto come panel test [1, 2]. Recentemente, il metodo SPME-GC-FID è stato proposto come metodo

strumentale adeguato per supportare la valutazione sensoriale attraverso la determinazione dei composti volatili (VOCs) [3]. Questo metodo, messo a punto nel progetto europeo OLEUM, nel quale tutte le variabili sono state definite ed armonizzate, è stato testato da tre laboratori nell'ambito di una validazione interlaboratorio per quantificare i VOCs maggiormente responsabili degli attributi sensoriali percepibili. Lo studio di validazione è stato applicato ad ognuno dei composti volatili considerati, così da avere un'informazione individuale delle prestazioni ottenibili per ogni analita.
Con il medesimo obiettivo, nel lavoro presente, cinque laboratori partecipanti al progetto OLEUM, hanno sviluppato una valutazione interlaboratorio del protocollo condiviso SPME-GC-MS. Per la validazione, come precedentemente fatto nel caso del metodo con il rivelatore FID, ogni laboratorio ha applicato le stesse procedure analitiche sugli stessi campioni, così da rendere i risultati ottenuti comparabili [3].

RISULTATI

Il metodo SPME-GC-MS, per la determinazione dei VOCs maggiormente responsabili dell'attributo di fruttato e dei principali difetti sensoriali di oli di oliva vergini, ha evidenziato una linearità (R^2 > 0,94) ed una ripetibilità (media della deviazione standard relativa, RSD% = 7,60%) soddisfacenti (Tabella).
I risultati della riproducibilità si sono dimostrati essere non dipendenti dal tipo di molecola.
I valori più contenuti di RSD% sono stati registrati per il (*Z*)-3-esenil acetato (19,19%), l'1-esanolo (13,26%) e l'acido acetico (17,47%). I limiti di quantificazione erano < 0,07 mg/kg per tutti i composti eccetto per la (*E*)-2-decenale e l'acido pentanoico. Lo studio comparativo di diversi metodi di quantificazione ha messo in rilievo come la correzione delle curve di calibrazione usando lo standard interno standard porti ad un risultato leggeremente peggiore in termini di ripetibilità ma ad una migliore accuratezza e riproducibilità. Questo studio si può considerare come una intervalidazione preliminare ad un processo di validazione completo.

Tabella I - Linearità espressa come R^2 (media e deviazione standard di cinque laboratori) calcolata dale curve di calibrazione usate nei metodi di quantificazione 1 e 2 (QM1, QM2) per i 18 composti volatili. Ripetibilità espressa come valore RSD% medio.

Composti volatili	Linearità (R^2)		RSD% (Media±SD)		
	QM1	QM2	QM1	QM2	QM3
Ottano	0.996±0.003	0.966±0.038[a]	6.77±4.33[c]	7.95±4.11	6.47±4.91
Etil acetato	0.982±0.023[a]	0.906±0.078[a]	6.99±3.49	4.77±0.21	5.75±4.02
Etanolo	0.984±0.011[a]	0.953±0.047[a]	9.51±2.72	6.21±2.14	6.52±1.94
Etill propanoato	0.994±0.008	0.939±0.053[a]	15.27±15.87[c]	15.55±15.63	15.13±17.34
Esanale	0.996±0.003	0.980±0.021	5.49±3.67	4.84±2.00	4.53±1.94
3-Metil-1-butanolo	0.996±0.002	0.941±0.068	5.09±1.80	5.63±2.58	2.88±2.44
(*E*)-2-Esenale	0.990±0.009[b]	0.994±0.007[b]	4.15±1.74	2.99±0.40	2.21±1.30
(*Z*)-3-Esenil acetato	0.987±0.012[b]	0.992±0.006[b]	5.23±0.55[e]	4.86±0.84[d]	3.11±0.61[ef]
(*E*)-2-Eptenale	0.976±0.027[b]	0.997±0.001	5.38±0.76	4.75±4.23	3.31±3.61
6-Metill-5-epten-2-one	0.975±0.025[b]	0.997±0.001	5.05±1.17	5.82±0.89	4.40±0.07
1-Esanolo	0.993±0.006	0.992±0.005	3.89±1.46	4.12±0.72[f]	2.39±0.34[f]
Nonanale	0.976±0.024	0.990±0.007	11.84±7.33[c]	9.89±3.96	7.36±9.39
1-Otten-3-olo	0.983±0.019	0.993±0.005	6.98±1.59	5.40±0.98	5.84±3.03
(*E,E*)-2,4-Esadienale	0.975±0.027	0.997±0.002	8.51±2.99	4.20±0.72	6.79±5.13
Acido acetico	0.993±0.005	0.989±0.011	7.87±0.47[d]	3.48±2.59[d]	5.48±3.09
Acido propanoico	0.983±0.028[b]	0.995±0.005	5.70±0.19[d]	2.35±1.56[d]	3.32±2.08
(*E*)-2-Decenale	0.942±0.057[b]	0.966±0.025[b]	17.23±5.08[e]	12.00±2.77	13.86±5.10[e]
Acido pentanoico	0.969±0.032[b]	0.993±0.008[b]	5.83±0.27[d]	3.17±0.58[d]	2.83±1.86

[a] Saturazione ad alta concentrazione evidenziata da alcuni laboratori coinvolti. [b] Sensibilità più bassa (pendenza inferiore) a basse concentrazioni evidenziata da alcuni laboratori coinvolti. [c] Un outlier è stato rimosso (Grubbs test p<0.05). [d] Differenza significativa (p<0.05) fra QM1 e QM2. [e] Differenza significativa (p<0.05) fra QM1 e QM3. [f] Differenza significativa (p<0.05) fra QM2 e QM3.

BIBLIOGRAFIA

[1] International Olive Council (IOC). Sensory analysis of olive oil method for the organoleptic assessment of virgin olive oil. IOOC/T.20/Doc. no. 3 (1987).
[2] European Commission Regulation. On the characteristics of olive oil and olive residue oil and on the relevant methods of analysis, and subsequent amendments. Official Journal of European Community, 11(L248), 1-102, 2568/91 (1991).

[3] E. Casadei, E. Valli, R. Aparicio-Ruiz, C. Ortiz-Romero, D.L. García-González, S. Vichi, B. Quintanilla-Casas, A. Tres, A. Bendini, T. Gallina Toschi, Peer inter-laboratory validation study of a harmonized SPME-GC-FID method for the analysis of selected volatile compounds in virgin olive oils. Food Control 123, 107823 (2021).

Questo abstract è basato sull'articolo pubblicato R. Aparicio-Ruiz, C. Ortiz Romero, E. Casadei, D.L. García-González, M. Servili, R. Selvaggini, F. Lacoste, J. Escobessa, S. Vichi, B. Quintanilla-Casas, P.A. Golay, P. Lucci, E. Moret, E. Valli, A. Bendini, T. Gallina Toschi, Collaborative peer validation of a harmonized SPME-GC-MS method for analysis of selected volatile compounds in virgin olive oils. Food Control (2022) https://doi.org/10.1016/j.foodcont.2021.108756