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Development of a Certified Reference Material for Virgin Olive Oil

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Abstract

This paper presents the results of the development of a Certified Reference Material (CRM) for virgin olive oil, focusing on the properties of volumetric acidity and fatty acid composition. The production of a CRM involves a series of stages, including production planning, preparation of the candidate material, homogeneity study, stability study, characterization, value assignment, and post-certification monitoring of the CRM. For the preparation of this CRM, commercial oil from the same production batch was used. The characterization of the properties was carried out following the approach outlined in section 7.12.3 C of the ISO 17034:2016 standard, utilizing a network of competent laboratories. The homogeneity study was assessed according to the ISO 35:2017 guideline. Short-term or transport stability was determined through an isochronous study at different temperatures, while long-term stability was evaluated monthly over a period of five months. The uncertainty of each property was calculated by considering the components of characterization, homogeneity, and short- and long-term stability. Subsequently, post-certification monitoring of the properties was conducted over a 15-month period. This work significantly contributes to enhancing the quality of measurements in the olive oil sector and promotes regional excellence in production.

Keywords: certified reference material, metrological traceability, olive oil, bias estimation, measurement uncertainty

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Desarrollo de un material de referencia certificado de aceite de oliva virgen

Resumen

En este trabajo se presentan los resultados del desarrollo de un material de referencia certificado (MRC) de aceite de oliva virgen para las propiedades de acidez libre y composición de ácidos grasos. La producción de un MRC conlleva una serie de etapas que abarcan el planeamiento de la producción, preparación del material candidato, estudio de homogeneidad, estudio de estabilidad, caracterización, asignación de valor y monitoreo postcertificación del MRC. Para la preparación de este MRC, se utilizó aceite comercial de un mismo lote de producción. La caracterización de las propiedades se llevó a cabo mediante el enfoque del punto 7.12.3 C de la norma ISO 17034:2016, utilizando una red de laboratorios competentes; el estudio de homogeneidad se evaluó según la guía ISO 35:2017. La estabilidad a corto plazo o de transporte se determinó mediante un estudio isócrono a distintas temperaturas, mientras que la estabilidad a largo plazo se evaluó mensualmente durante cinco meses. La incertidumbre de cada propiedad se calculó considerando las componentes de caracterización, homogeneidad y estabilidad a corto y largo plazo. Luego se realizó un monitoreo postcertificación de las propiedades durante un período de 15 meses. Este trabajo contribuye significativamente al fortalecimiento de la calidad de las mediciones en el sector olivícola y promueve la excelencia en la producción a nivel regional.

Palabras clave: material de referencia certificado, trazabilidad metrológica, aceite de oliva, estimación del sesgo, incertidumbre de medición

Desenvolvimento de um material de referência certificado para azeite de oliva virgem

Resumo

Este trabalho apresenta os resultados do desenvolvimento de um Material de Referência Certificado (MRC) para azeite de oliva virgem, com foco nas propriedades de acidez livre e composição de ácidos graxos. A produção de um MRC envolve uma série de etapas, incluindo planejamento da produção, preparação do material candidato, estudo de homogeneidade, estudo de estabilidade, caracterização, atribuição de valor e monitoramento pós-certificação do MRC. Para a preparação deste MRC, foi utilizado azeite comercial proveniente do mesmo lote de produção. A caracterização das propriedades foi realizada seguindo a abordagem descrita na seção 7.12.3 C da norma ISO 17034:2016, utilizando uma rede de laboratórios competentes. O estudo de homogeneidade foi avaliado de acordo com a diretriz ISO 35:2017. A estabilidade de curto prazo ou de transporte foi determinada por meio de um estudo isócrono em diferentes temperaturas, enquanto a estabilidade de longo prazo foi avaliada mensalmente durante um período de cinco meses. A incerteza de cada propriedade foi calculada considerando os componentes de caracterização, homogeneidade e estabilidade de curto e longo prazo. Posteriormente, foi realizado o monitoramento pós-certificação das propriedades ao longo de um período de 15 meses. Este trabalho contribui significativamente para a melhoria da qualidade das medições no setor de azeite de oliva e promove a excelência na produção em nível regional.

Palavras-chave: material de referência certificado, rastreabilidade metrológica, azeite de oliva, estimativa de viés, incerteza de medição

1. Introduction

In Argentina, olive oil production is one of the main drivers of regional economic activity, encompassing the provinces of Buenos Aires, Catamarca, Córdoba, Chubut, La Rioja, Mendoza, Neuquén, Río Negro, San Juan, San Luis, Santiago del Estero, and Salta. According to data from The National Institute of Statistics and Censuses of the Argentine Republic (INDEC), in the first half of 2024, olive oil exports totaled 117 million dollars, equivalent to 0.3% of the country's total exports, with a year-on-year increase of 57.5%. The main destinations were member countries of MERCOSUR, USMCA, and the European Union (Instituto Nacional de Estadística y Censos, 2024).

At the regional level, Brazil, one of the top 10 global consumers, stands out as the primary buyer, while Chile and Peru are notable producers, with a significant portion of their production destined for the international market (Ministerio de Economía, 2024).

In response to the growing demands of laboratories certified under quality standards, the calibration of equipment, validation of methods, and estimation of their uncertainties are essential requirements for optimal performance. The use of Certified Reference Materials (CRMs) is indispensable for achieving these objectives (Leiva-Guzmán, 2006).

The establishment of a CRM for olive oil in the region is essential for ensuring the accuracy and reliability of measurements within the olive oil sector. Furthermore, it contributes to improving production quality at the national level. Although the availability of Reference Materials (RMs) and CRMs on the market has increased in recent years, the supply of these materials in specific matrices tailored to the needs of the oil industry in the region remains highly limited and does not fully meet industry requirements. In this context, the National Institute of Industrial Technology (INTI), recognized as the National Metrology Institute (NMI) and authorized to produce CRMs, developed, in collaboration with the Argentine Interlaboratory Service (SAI), a CRM for virgin olive oil using a commercial oil sample, specifically for the properties of free acidity and fatty acid composition.

A CRM is a material characterized using a valid metrological procedure for one or more specific properties, accompanied by a certificate that provides the specified property value, its associated uncertainty, and a statement of metrological traceability (International Organization for Standardization [ISO], 2016).

CRMs are used for a wide range of applications in various fields. Their properties are sufficiently homogeneous and well-established to be used in process monitoring, statistical control, calibration of laboratory instruments, validation of test methods, laboratory proficiency evaluation, value assignment to other materials, and for ensuring metrological traceability (ISO, 2016).

The production of a CRM involves multiple stages, including production planning, candidate batch preparation, homogeneity assessment, stability study, characterization and value assignment, and post-certification monitoring.

2. Materials and Methods

2.1 Analytical Techniques Employed in the Production of the CRM

The quantification of free acidity was carried out following the ISO 660:2020 standard (Method 9.1: “Cold solvent method using an indicator”) (ISO, 2020). For this method, a 10 ml Bang-type microburette (Class A, division 1/50, IVA Argentina) was used, with potassium hydrogen phthalate (Cetripur® Merck, purity: 100.00% ± 0.05% (k=2), traceable to NIST SRM®) as the primary standard.

The fatty acid composition was determined according to the protocols of ISO 12966-2:2017 (ISO, 2017a) and ISO 12966-4:2015 (ISO, 2015a). A gas chromatograph (Perkin Elmer, model: Clarus 580) equipped with a flame ionization detector (FID) was employed. The system was fitted with a 100 m long, 0.25 mm diameter fused silica capillary column, coated with a 0.20 µm thick poly (biscyanopropyl siloxane) stationary phase (Supelco SP-2560), selected for its resolution of geometric isomers (cis/trans). The injector was a Split/Splitless type, and a 0.63 µm thick syringe was used.

The operating conditions for the chromatographic run were as follows:

- Injector temperature: 240 °C

- Detector temperature: 280 °C
- Oven temperature: 120 to 240 °C, at a rate of 4 °C/min, holding at 240 °C for 7 minutes
- Carrier gas: Hydrogen (Column head pressure at 220 kPa)
- Detector gases: Hydrogen 40 ml/min – Air 450 ml/min
- Flow: Linear velocity of 20 cm/s (Approximate flow of 1.0 ml/min)
- Split ratio: 1:100
- Injection volume: 1 µl (equivalent to 15-20 µg of methyl esters)

The standards used for identification were:

- FAME Mix 37 (Supelco, CRM47885)
- Linoleic Acid Methyl Ester Mix (Supelco, CRM47791)
- Linolenic Acid Methyl Ester Isomer Mix (Supelco, CRM47792)

Chromatographic analysis was performed considering the identification and quantification of all fatty acids present, including trans isomers. Each fatty acid was expressed as the sum of its positional and geometric isomers. Concentrations below 0.05 g/100 g of methyl esters were not reported in the CRM certificate.

2.2 Preparation of the Candidate CRM

The candidate CRM for virgin olive oil was prepared using a commercial oil sample from the same production batch. The authenticity of the sample was verified through the analysis of the fatty acid profile and free acidity to ensure that these properties fell within the ranges specified for virgin olive oil under the International Olive Council (COI) trade standard (International Olive Council, 2021).

Olive oil sample was placed in a 50-liter stainless steel container and subjected to mechanical agitation during 5 minutes, avoiding the formation of air bubbles. These procedures were performed to ensure proper homogenization and prevent oxidation risk.

The oil sample was slowly dispensed into bottles, ensuring that no air space remained within each container. This procedure was meticulously performed to avoid the introduction of air, which is critical for preserving the oil's quality. The bottles were then hermetically sealed, numbered and labeled sequentially in ascending order according to the filling sequence. Amber-colored PET bottles with a capacity of approximately 125 ml, equipped with tamper-evident screw caps, were used for the packaging.

The entire procedure was conducted in a laboratory under controlled ambient temperature conditions, ensuring the stability of the candidate CRM. To ensure impartiality and representativeness of the samples, the SAI randomly selected the samples used for evaluating homogeneity, stability, and batch monitoring.

The laboratory recorded the use of each sample in a designated form. The candidate CRM batch was stored under refrigeration to preserve its properties until required for use.

2.3 Homogeneity

The objective of the homogeneity evaluation is to statistically ensure that the property values of all units within the batch remain consistent.

For the homogeneity analysis, 10 samples of the produced material were selected using simple random sampling. The homogeneity of the properties of free acidity and fatty acid profile was evaluated in the 10 selected

samples under repeatability conditions, duplicate in accordance with the guidelines of ISO 35:2017, section 7.7.3 (ISO, 2017c).

The acceptance criterion for homogeneity was based on a one-way analysis of variance (ANOVA). To consider the samples homogeneous, the critical F-value (obtained from the F-distribution table) must be smaller than the F-value derived from the determination data. If this criterion is not met, it is inferred that there are effects associated with a lack of homogeneity between samples. To determine whether these effects are significant, the criterion of sufficient homogeneity is applied: the between-sample variance (sm^2) is compared with the critical value c defined in ISO 13528:2018 (section B.2.3.b) (ISO, 2015c).

This criterion ensured that the observed variations between the samples were not significant, thereby confirming the homogeneity of the batch.

2.4 Stability

The objective of the stability study is to ensure that the value of each property remains consistent with the certified value throughout the material's period of use.

Short-term stability was evaluated through an isochronous study conducted in an oven at a reference temperature of T=30 ± 1 °C for two weeks. Two bottles were selected for each exposure time: 0, 7, and 15 days following the exposure period, all units were stored at −18°C ± 2°C. The properties of the samples, subjected to different exposure times, were then measured in duplicate.

The evaluation of the short-term stability study results was conducted following ISO 35:2017, section 8.5 (ISO, 2017c), with linear regression employed as the data analysis method. For the long-term stability study, two samples in duplicate were evaluated monthly for the first 5 months under ambient temperatures ranging from 20 to 25 °C and storage temperatures between 4 and 8 °C, using a real-time study approach. The shelf life of the CRM was established as the maximum period during which the material maintains stability within an acceptable confidence interval. An extrapolation was performed by fitting a linear regression to the obtained data, and it was concluded that the material would remain stable for 2 years.

Subsequently, post-certification stability monitoring was conducted. Following the same criteria applied in the short- and long-term stability studies, samples were analyzed quarterly over 15-month period starting from the date of CRM certification. This ongoing monitoring of the CRM allowed to evaluate potential deviations in the certified property values and confirmed that the CRM remained stable. Additionally, the monitoring results enabled the extension of the material's validity period.

The samples chosen for all stability studies were selected randomly.

2.5 Characterization

The characterization of a CRM involves the determination of the values or attributes of its properties as part of the production process (ISO, 2015b). Similarly, IUPAC defines the characterization of a CRM as the determination of one or more values of physical, chemical, biological, or technological properties that are relevant to its intended final use (IUPAC Compendium of Analytical Nomenclature).

In the development of this CRM, the characterization of the properties was conducted following the approach outlined in section 7.12.3 C of the ISO 17034:2016 standard (ISO, 2016), which involves characterizing the operationally defined measurands through a network of competent laboratories. Each property of interest was characterized independently with appropriate traceability and sufficient reliability.

The characterization data were obtained from an intercomparison involving four laboratories from the following INTI departments: Análisis Instrumental; Red de Laboratorios (REDELAC); Servicios Analíticos Región Pampeana; and Servicios Analíticos Cuyo. The selection of these laboratories was based on their performance evaluation in the proficiency test "Analysis of Edible Vegetable Oils PRA-01/2021". Additionally, a technical questionnaire was administered to harmonize measurement criteria and ensure their competence in accordance with the requirements of the ISO/IEC 17025:2017 standard (ISO, 2017b).

Each laboratory received three coded samples from the same production batch, which were tested in duplicate by two operators using two operationally defined and independent methodologies.

Furthermore, they were provided with a control sample from the COI/CHEM 2021 proficiency test, organized by COI, in which INTI participates annually. This sample enabled the verification of the measurements systems of each competent laboratory and ensured the repeatability of the measurements.

2.6 Value Assignment and Uncertainty Calculation

Value assignment is the process by which the property or attribute values of a CRM, obtained through characterization, are consolidated and documented in the accompanying CRM documentation.

The value of each assigned property for this CRM was determined using procedures that consider experimental designs and statistical techniques. The value assignment of the properties was carried out by calculating the unbiased mean of the measurand values, operationally defined using data collected from the homogeneity study and the intercomparison results from competent laboratories.

Each of these property values was associated with a standard uncertainty, which was calculated using the uncertainty components derived from characterization, homogeneity, and short- and long-term stability studies. The reported expanded uncertainty was calculated by multiplying the combined standard uncertainty by a coverage factor k = 2, corresponding to an approximate confidence level of 95% under a normal distribution.

2.7 Metrological Traceability

The metrological traceability of the certified values was established in accordance with the requirements outlined in the ISO/IEC 17025:2017 standard (ISO, 2017b), specifically in section 6.5.3, through the use of internationally recognized reference measurement methods. These methods were implemented by competent laboratories selected by INTI (ISO, 2016). To ensure the measurements quality, INTI laboratories operate in accordance with the guidelines set forth in the ISO/IEC 17025 standard. This standard establishes the general requirements for the technical competence of testing laboratories and provides a robust framework for quality management and technical proficiency. The test methods employed have been rigorously validated and are subject to periodic internal and external audits, conducted by Organismo Argentino de Acreditación (OAA).

Additionally, the measurement equipment is regularly calibrated using certified reference standards with metrological traceability. To monitor the stability and precision of processes over time, control charts are employed, enabling the detection and correction of any potential deviations in the measurements.

In summary, the combination of calibrated equipment, certified reference standards, compliance with the ISO/IEC 17025 standard, internal and external audits, control charts, accredited methods, and interlaboratory participation ensures the quality and traceability of the measurements. This guarantees the reliability and trustworthiness of the results obtained.

3. Results and Discussion

3.1 Homogeneity

Table 1 shows F and $F_{critical}$ values obtained from the ANOVA analysis. It can be observed that, for all properties except free acidity and lignoceric acid (for which the second criterion of sufficient homogeneity mentioned in section 2.3 was applied), the acceptance criterion $F < F_{critical}$ is met.

Table 1. Uncertainty component associated with the homogeneity of the samples

Property	F	$F_{critical}$
Hexadecanoic acid (palmitic acid, C16:0)	1.53	3.02
cis-9-Hexadecenoic acid (palmitoleic acid, C16:1, n-7) + positional and geometric isomers	0.96	3.02
Heptadecanoic acid (margaric acid, C17:0)	0.99	3.02
cis-10-Heptadecenoic acid (margaroleic acid, C17:1, n-7)	1.13	3.02
Octadecanoic acid (stearic acid, C18:0)	0.94	3.02
cis-9-Octadecenoic acid (oleic acid, C18:1, n-9) + positional and geometric isomers	1.06	3.02
cis, cis-9,12-Octadecadienoic acid (linoleic acid, C18:2, n-6) + positional and geometric isomers	0.92	3.02
cis, cis, cis-9,12,15-Octadecatrienoic acid (linolenic acid, C18:3, n-3) + positional and geometric isomers	1.23	3.02
Eicosanoic acid (arachidic acid, C20:0)	0.81	3.02
cis-11-Eicosenoic acid (gondoic acid, C20:1, n-9)	0.43	3.02
Docosanoic acid (behenic acid, C22:0)	1.34	3.02

Table 2 indicates the uncertainty component associated with the homogeneity of the samples (u_{homog}) for each of the properties.

Table 2. Uncertainty component associated with the homogeneity of the samples

	Property	U_{homog}
Fatty Acids (g/100g methyl esters)	Hexadecanoic acid (palmitic acid, C16:0)	0.010503
	cis-9-Hexadecenoic acid (palmitoleic acid, C16:1, n-7) + positional and geometric isomers	0.001045
	Heptadecanoic acid (margaric acid, C17:0)	0.000041
	cis-10-Heptadecenoic acid (margaroleic acid, C17:1, n-7)	0.000369
	Octadecanoic acid (stearic acid, C18:0)	0.000879
	cis-9-Octadecenoic acid (oleic acid, C18:1, n-9) + positional and geometric isomers	0.009095
	cis, cis-9,12-Octadecadienoic acid (linoleic acid, C18:2, n-6) + positional and geometric isomers	0.006201
	cis, cis, cis-9,12,15-Octadecatrienoic acid (linolenic acid, C18:3, n-3) + positional and geometric isomers	0.001329
	Eicosanoic acid (arachidic acid, C20:0)	0.001115
	cis-11-Eicosenoic acid (gondoic acid, C20:1, n-9)	0.002353
	Docosanoic acid (behenic acid, C22:0)	0.000587
	Tetracosanoic acid (lignoceric acid, C24:0)	0.000807
Free Acidity (g oleic acid/100 g)		0.004303

3.2 Stability

Using the statistical tool of linear regression for both short- and long-term stability studies, it was demonstrated that for all properties, the slope of the regression line was statistically negligible. This confirms that the acceptance criterion for the stability of the certified properties was met.

The results of the long- and short-term stability studies are presented in Tables 3 and 4.

3.3 Short-Term Stability

Table 3. Short-term stability table

Time	T0	T1 (at 7 days)	T2 (at 15 days)
Free Acidity			
Free Acidity (g oleic acid/100 g)	0.36	0.36	0.36
Fatty Acids (g/100 g methyl esters)			
Hexadecanoic acid (palmitic acid, C16:0)	14.56	14.64	14.61
cis-9-Hexadecenoic acid (palmitoleic acid, C16:1, n-7) + positional and geometric isomers	1.46	1.45	1.45
Heptadecanoic acid (margaric acid, C17:0)	0.09	0.09	0.09
cis-10-Heptadecenoic acid (margaroleic acid, C17:1, n-7)	0.21	0.21	0.22
Octadecanoic acid (stearic acid, C18:0)	1.96	1.95	1.95
cis-9-Octadecenoic acid (oleic acid, C18:1, n-9) + positional and geometric isomers	68.64	68.58	68.64
cis, cis-9,12-Octadecadienoic acid (linoleic acid, C18:2, n-6) + positional and geometric isomers	11.66	11.65	11.62
cis, cis, cis-9,12,15-Octadecatrienoic acid (linolenic acid, C18:3, n-3) + positional and geometric isomers	0.62	0.62	0.62
Eicosanoic acid (arachidic acid, C20:0)	0.35	0.35	0.36
cis-11-Eicosenoic acid (gondoic acid, C20:1, n-9)	0.31	0.31	0.32
Docosanoic acid (behenic acid, C22:0)	0.11	0.10	0.10
Tetracosanoic acid (lignoceric acid, C24:0)	0.06	0.06	0.06

3.4 Long-Term Stability

Table 4. Long-term stability table

Time	T0	T1	T2	T3	T4	T5
Free Acidity						
Free Acidity (g oleic acid/100 g)	0.33	0.33	0.36	0.36	0.36	0.36
Fatty Acids (g/100 g methyl esters)						
Hexadecanoic acid (palmitic acid, C16:0)	14.74	14.72	14.76	14.71	14.70	14.71
cis-9-Hexadecenoic acid (palmitoleic acid, C16:1, n-7) + positional and geometric isomers	1.46	1.46	1.48	1.46	1.46	1.48
Heptadecanoic acid (margaric acid, C17:0)	0.09	0.09	0.10	0.09	0.09	0.10
cis-10-Heptadecenoic acid (margaroleic acid, C17:1, n-7)	0.21	0.20	0.22	0.21	0.21	0.21
Octadecanoic acid (stearic acid, C18:0)	1.945	1.94	1.95	1.94	1.95	1.96
cis-9-Octadecenoic acid (oleic acid, C18:1, n-9) + positional and geometric isomers	68.50	68.51	68.43	68.58	68.56	68.50
cis, cis-9,12-Octadecadienoic acid (linoleic acid, C18:2, n-6) + positional and geometric isomers	11.63	11.63	11.64	11.60	11.61	11.61
cis, cis, cis-9,12,15-Octadecatrienoic acid (linolenic acid, C18:3, n-3) + positional and geometric isomers	0.63	0.62	0.62	0.61	0.62	0.62
Eicosanoic acid (arachidic acid, C20:0)	0.35	0.35	0.36	0.35	0.35	0.35
cis-11-Eicosenoic acid (gondoic acid, C20:1, n-9)	0.3	0.31	0.31	0.29	0.31	0.32
Docosanoic acid (behenic acid, C22:0)	0.10	0.10	0.10	0.10	0.10	0.11
Tetracosanoic acid (lignoceric acid, C24:0)	0.05	0.06	0.06	0.06	0.06	0.06

3.5 Assignment of Certified Values

The certified values for fatty acids are reported in Tables 5 and 7, expressed as the mass percentage of fatty acid methyl esters. The certified value for free acidity is reported in Tables 6 and 8, expressed as the mass percentage of grams of oleic acid per 100 grams of sample.

Table 5. Certified values for fatty acid composition: First version of the certificate for the virgin olive oil reference material INTI-CRM 031-001.

Certified values	
Property	**Value and Uncertainty**
Hexadecanoic acid (palmitic acid, C16:0)	14.55 ± 0.30 (g/100 g)
cis-9-Hexadecenoic acid (palmitoleic acid, C16:1, n-7) + positional and geometric isomers: cis-7-Hexadecenoic acid	1.45 ± 0.03 (g/100 g)
Heptadecanoic acid (margaric acid, C17:0)	0.09 ± 0.01 (g/100 g)
cis-10-Heptadecenoic acid (margaroleic acid, C17:1, n-7)	0.21 ± 0.01 (g/100 g)
Octadecanoic acid (stearic acid, C18:0)	1.95 ± 0.02 (g/100 g)
cis-9-Octadecenoic acid (oleic acid, C18:1, n-9) + positional and geometric isomers: trans-9-Octadecenoic acid (elaidic acid), cis-11-Octadecenoic acid (cis-vaccenic acid)	68.55 ± 0.44 (g/100 g)
cis, cis-9,12-Octadecadienoic acid (linoleic acid, C18:2, n-6) + positional and geometric isomers: trans, cis-9,12-Octadecadienoic acid, cis, trans-9,12-Octadecadienoic acid, trans, trans-9,12-Octadecadienoic acid	11.68 ± 0.10 (g/100 g)
cis, cis, cis-9,12,15-Octadecatrienoic acid (linolenic acid, C18:3, n-3) + positional and geometric isomers: trans, cis, cis-9,12,15-Octadecatrienoic acid, cis, trans, cis-9,12,15-Octadecatrienoic acid, cis, cis, trans-9,12,15-Octadecatrienoic acid, cis, trans, trans-9,12,15-Octadecatrienoic acid, trans, cis, trans-9,12,15-Octadecatrienoic acid, trans, trans, cis-9,12,15-Octadecatrienoic acid, trans, trans, trans-9,12,15-Octadecatrienoic acid	0.64 ± 0.02 (g/100 g)
Eicosanoic acid (arachidic acid, C20:0)	0.35 ± 0.02 (g/100 g)
cis-11-Eicosenoic acid (gondoic acid, C20:1, n-9)	0.31 ± 0.02 (g/100 g)
Docosanoic acid (behenic acid, C22:0)	0.10 ± 0.01 (g/100 g)
Tetracosanoic acid (lignoceric acid, C24:0)	0.05 ± 0.01 (g/100 g)
Measurand expressed as grams of the listed fatty acid methyl ester per 100 grams of total methyl esters.	

The reported expanded uncertainty was calculated by multiplying the combined standard uncertainty by a coverage factor k = 2, which corresponds to an approximate confidence level of 95% under a normal distribution.

Table 6. Certified values for free acidity: First version of the certificate for the virgin olive oil reference material INTI-CRM 031-001.

Certified Value	
Property	**Value and Uncertainty**
Free Acidity (expressed as oleic acid)	0.34 ± 0.02 (g/100 g)

The reported expanded uncertainty was calculated by multiplying the combined standard uncertainty by a coverage factor k = 2, which corresponds to an approximate confidence level of 95% under a normal distribution.

3.6 Post-Certification Monitoring

The certified property values, including post-certification monitoring, are presented in Tables 7 and 8.

Table 7. Certified values for fatty acid composition: Second version of the certificate for the virgin olive oil reference material INTI-CRM 031-001.

Certified values	
Property	**Value and Uncertainty**
Hexadecanoic acid (palmitic acid, C16:0)	14.55 ± 0.31 (g/100 g)
cis-9-Hexadecenoic acid (palmitoleic acid, C16:1, n-7) + positional and geometric isomers: cis-7-Hexadecenoic acid	1.45 ± 0.03 (g/100 g)
Heptadecanoic acid (margaric acid, C17:0)	0.09 ± 0.01 (g/100 g)
cis-10-Heptadecenoic acid (margaroleic acid, C17:1, n-7)	0.21 ± 0.01 (g/100 g)
Octadecanoic acid (stearic acid, C18:0)	1.95 ± 0.03 (g/100 g)
cis-9-Octadecenoic acid (oleic acid, C18:1, n-9) + positional and geometric isomers: trans-9-Octadecenoic acid (elaidic acid), cis-11-Octadecenoic acid (cis-vaccenic acid)	68.55 ± 0.41 (g/100 g)
cis, cis-9,12-Octadecadienoic acid (linoleic acid, C18:2, n-6) + positional and geometric isomers: trans, cis-9,12-Octadecadienoic acid, cis, trans-9,12-Octadecadienoic acid, trans, trans-9,12-Octadecadienoic acid	11.68 ± 0.11 (g/100 g)
cis, cis, cis-9,12,15-Octadecatrienoic acid (linolenic acid, C18:3, n-3) + positional and geometric isomers: trans, cis, cis-9,12,15-Octadecatrienoic acid, cis, trans, cis-9,12,15-Octadecatrienoic acid, cis, cis, trans-9,12,15-Octadecatrienoic acid, cis, trans, trans-9,12,15-Octadecatrienoic acid, trans, cis, trans-9,12,15-Octadecatrienoic acid, trans, trans, cis-9,12,15-Octadecatrienoic acid, trans, trans, trans-9,12,15-Octadecatrienoic acid	0.64 ± 0.03 (g/100 g)
Eicosanoic acid (arachidic acid, C20:0)	0.35 ± 0.02 (g/100 g)
cis-11-Eicosenoic acid (gondoic acid, C20:1, n-9)	0.31 ± 0.02 (g/100 g)
Docosanoic acid (behenic acid, C22:0)	0.10 ± 0.01 (g/100 g)
Tetracosanoic acid (lignoceric acid, C24:0)	0.05 ± 0.01 (g/100 g)
Measurand expressed as grams of the listed fatty acid methyl ester per 100 grams of total methyl esters.	

The reported expanded uncertainty was calculated by multiplying the combined standard uncertainty by a coverage factor k = 2, which corresponds to an approximate confidence level of 95% under a normal distribution.

Table 8. Certified values for free acidity: Second version of the certificate for the virgin olive oil reference material INTI-CRM 031-001.

Certified Value	
Property	**Value and Uncertainty**
Free Acidity (expressed as oleic acid)	0.34 ± 0.04 (g/100 g)

The reported expanded uncertainty was calculated by multiplying the combined standard uncertainty by a coverage factor k = 2, which corresponds to an approximate confidence level of 95% under a normal distribution.

4. Conclusions

The development of the first batch of the CRM for virgin olive oil was completed as planned, yielding certified values for fatty acid composition and free acidity. These values are accompanied by comprehensive information on their metrological traceability and associated uncertainties.

The development of this CRM represents a major step forward in strengthening the quality of analytical measurements, thereby contributing significantly to ensuring reliable and internationally comparable results and to promoting harmonization across the sector.

INTI intends to repeat the production of this CRM, which will further enhance the precision and consistency of measurements in the industry. This ongoing effort will ensure the quality and reliability of results, consolidating INTI's position as a leader in applied metrology for the olive oil industry.

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Transparency of Data

The entire data set that supports the results of this study was published in the article itself.

Author contribution statement

	LS	SE	PB	CS	MS	MC	GM	SF	AC	AS	FR	MCS	RB	SA	EAK	PG	GR	MV
Conceptualization	■	■			■	■	■								■	■		
Data curation	■	■				■												
Formal analysis							■	■	■									
Funding acquisition															■	■		
Investigation	■	■	■	■						■	■	■						
Methodology	■	■		■	■	■	■						■	■		■		
Project administration	■					■										■		
Resources															■	■		
Software								■	■								■	
Supervision														■	■	■		
Validation	■	■	■		■	■	■	■	■									
Visualization	■	■	■			■												
Writing – original draft	■		■															
Writing – review and editing	■	■	■		■	■	■										■	■

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