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ORIGINAL ARTICLE

Metrological framework for the development of plant-based certified reference materials. Part I

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Abstract: Certified reference materials constitute a fundamental principle of reliable measurements across scientific, industrial, and regulatory domains, as they provide the basis for the formal demonstration of metrological traceability and result comparability. Unfortunately, the availability of certified reference materials for complex plant matrices, particularly fruit or leaves tissues, remains limited. This study presents the scenario for the development of MultiBio CRM, a strawberry-based certified reference material pair comprising leaves and fruit, which reflects compositional characteristics typical of plant materials cultivated under Polish environmental and agricultural conditions. The study further demonstrates a complete, ISO-aligned workflow for the production of plant-based reference materials. The production strategy was designed in accordance with ISO 17034, ISO 33405, and related international requirements for reference material production. It encompasses matrix selection, controlled cultivation and sampling, pre-treatment, drying or lyophilization, particle-size reduction, homogenization, stabilization, and certification. Material homogeneity and stability were verified using statistically designed studies, and uncertainty contributions were evaluated following internationally accepted guidelines. The resulting certified reference materials represent robust, internationally compliant plant matrices and illustrate a comprehensive metrological approach to reference material development. This work addresses a significant gap in existing certified reference material portfolios and supports reliable, traceable measurements in food-safety and environmental analysis.

Keywords: Certified reference materials, plant-based reference materials, metrological traceability, food safety analysis, environmental monitoring, strawberry matrix

1 Introduction

Certified reference materials (CRMs) play a fundamental role in ensuring the traceability, comparability, and long-term consistency of measurement results (Bulska et al., 2026). In chemical and biological analysis, instrument signals do not inherently possess metrological meaning unless they can be related to recognized reference values. Certified reference materials fulfil this function by serving as material carriers of property values that are traceable to the International System of Units (SI) and accompanied by stated measurement uncertainties. Through their certified values, CRMs enable the transfer of metrological traceability from primary standards and reference procedures to analytical results obtained in routine laboratory, thereby providing a direct link between measurement units and the results for test analytical samples.

Certified reference materials represent the highest confidence level among reference materials. Their property values are assigned using validated measurement procedures and are documented in certificates that include uncertainty estimates and explicit statements of traceability. Consequently, CRMs provide a valid basis for instrument calibration, method validation, verification of analytical performance, and estimation of measurement uncertainty (Bulska et al., 2026). Their systematic use is essential to ensure that measurement results are accurate, comparable across laboratories, and suitable for regulatory, industrial, and scientific decision-making. More broadly, the use of structured

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quality-assurance protocols and metrologically controlled workflows is essential for ensuring the validity and comparability of analytical results across complex measurement systems (Gawor & Bulska, 2023; Wryk et al., 2024).

International standards and guidance documents define the principles governing the production, documentation, characterization, and use of certified reference materials. ISO 17034:2016 (ISO 17034, 2016) specifies the competence requirements for reference material producers, including quality management, technical operations, and control of production processes. Fundamental terminology and definitions related to reference materials are currently established in ISO Guide 30:2015 (2015), which was reviewed and formally confirmed in 2024 and therefore remains valid at the time of this study. At the same time, this guide is expected to be replaced by the forthcoming International Standard ISO/DIS 33400 "Reference materials. Selected terms and definitions", which is currently under development and planned as the next generation of harmonized terminology in this field. More recent standards further strengthen and update existing requirements in specific areas of reference material production and use. ISO 33401:2024 (2024) defines detailed requirements for the content and structure of certificates, labels, and accompanying documentation for reference materials, while ISO 33405:2024 (2024) addresses methodological and technical aspects related to the characterization, assessment of homogeneity, and stability of reference materials. In addition, ISO 33403:2024 (2024) specifies requirements and provides practical recommendations for the appropriate selection, handling, and use of reference materials, thereby supporting their correct application throughout the measurement process. From the laboratory perspective, compliance with ISO/IEC 17025:2017 (2017) and ISO 15189:2022 (2022) requires the use of suitable CRMs to formally demonstrate metrological traceability and comparability of results. This requirement is closely linked to broader issues of competence, documentation, and quality-system implementation in accredited laboratories (Gawor et al., 2021). These standards also mandate method revalidation whenever changes in reference materials, matrices, or analytical conditions may influence measurement results, thereby reinforcing the central role of CRMs in regulated analytical practice.

Despite their central role in measurement science, fit-for-purpose certified reference materials are not available for all analytes, concentration ranges, or sample matrices, a limitation that is particularly pronounced for complex biological and environmental materials. The absence of an appropriate, matrix-matched CRM primarily affects the accuracy of measurement results, as it limits the ability to detect and correct systematic bias arising from matrix-dependent effects during sample preparation and instrumental measurement. Even when reference materials are available, incomplete matrix matching between the CRM and real samples may still lead to differences in analyte recovery, signal response, or chemical behaviour, thereby compromising result trueness. This issue is independent of the formal availability of a CRM and highlights that matrix commutability, rather than nominal matrix similarity, is a critical requirement for reliable bias control. As demonstrated by Wysocka (2021), the lack of suitable matrix-matched CRMs constitutes a major obstacle to achieving low combined measurement uncertainty in the determination of rare earth elements in natural waters, primarily due to increased uncertainty associated with bias estimation rather than analytical repeatability. In situations where appropriate CRMs are unavailable or insufficiently commutable, laboratories may apply alternative, scientifically justified approaches, such as the use of well-characterized non-certified reference materials, in-house quality control materials, standard addition methods, or participation in proficiency testing schemes. However, these approaches cannot fully replace CRMs for the formal demonstration of metrological traceability. Their use therefore necessitates careful method validation, explicit documentation of traceability assumptions, and conservative estimation of measurement uncertainty, as stipulated in ISO/IEC 17025:2017 and ISO 15189:2022 (ISO 17034, 2016; ISO/IEC 17025:2017, 2017; ISO 15189:2022, 2022).

Plant-based certified reference materials are of particular importance in agriculture (Trimmel et al., 2023; Marguí et al., 2005; Sapkota et al., 2005), food safety, pharmaceutical analysis (Queralt et al., 2005; Kalny et al., 2007), and environmental monitoring (ISO 33403:2024, 2024; ISO/IEC 17025:2017, 2017; ISO 15189:2022, 2022; Gawor et al., 2021; Wysocka, 2021; Trimmel et al., 2023; Marguí et al., 2005). In these fields, reliable and traceable measurements are critical for quality control, regulatory compliance, and consumer protection. Increasing environmental pressure related to anthropogenic contamination further reinforces the need for reference materials that realistically represent plant matrices. Toxic elements such as arsenic, cadmium, lead, and mercury are frequently detected in foodstuffs, drinking water, and ambient air, posing significant risks to public health (Rai et al., 2019; Danopoulos et al., 2020; Eerkes-Medrano et al., 2019; Oßmann et al., 2018; Gambino et al., 2022; Kosuth et al., 2018). At the same time, emerging contaminants, including microplastics, are receiving growing attention. Although their long-term health effects remain under investigation, existing evidence indicates that both classical toxic elements and emerging contaminants may adversely affect human health (El-Kady & Abdel-Wahhab, 2018; Prata, 2018). These challenges underscore the need for robust, traceable, and matrix-matched reference materials that reflect real plant systems. Recent CRM studies in food analysis similarly emphasize that matrix-based CRMs are required to reproduce matrix effects during sample preparation and measurement, while their availability remains limited for many analyte–matrix combinations (Lobsiger et al., 2023).

The production of certified reference materials for plant matrices follows internationally harmonized principles derived from long-standing metrological practice and formalized in ISO standards. An ISO-aligned workflow typically includes the selection of representative plant material, pre-treatment procedures such as drying or lyophilization, grinding, and homogenization to ensure bulk-material uniformity. Subsequent stages involve

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Table 1. Step-wise comparison of plant matrix CRMs (BARC, 2026; National Institute of Standards and Technology, 2022)

Step	NIST SRM 1515 (Apple Leaves)	BARC-D3201 (Black tea powder)	Notes
Sourcing	Cultivation/collection (bulk lot)	Purchase/cultivation; 3.6 kg batch	–
Pre-treatment	Drying, milling, 1 mm sieving of entire lot	Drying milling, 1 mm sieving of subset used for prelamination tests	Aligns lot before size refinement
Size refinement	(as applicable) jet milling and air classification to 75 µM (200 mesh)	Jet milling and air classification to 75 µM	Targets narrow PSD for homogeneity
Preliminary characterization	22 elements	15 elements	After dissolution
Targeted spiking	No spiking	4 elements (solutions): Pb, As, Cd, Hg	Followed by dry, mix, sieve
Preservation	^{60}Co γ-irradiation, 27.8 kGy	TBHQ added during enrichment	tert-butylhydroquinone (TBHQ)
Bottling	50 g per bottle	20 g per bottle, 146 units	Unit mass differs
Certification and QC	Homogeneity and stability, ILCs	Homogeneity and stability, ILCs	Leads to certification

preliminary multi element characterization and, where native analyte concentrations are too low to be analytically or legislatively relevant, targeted spiking to adjust their levels accordingly. The workflow is completed by bottling and packaging, supported by homogeneity and stability studies and external characterization through interlaboratory comparisons, which together form the basis for certification.

For plant matrices, additional preparative operations are often required to address their inherent heterogeneity and biological nature. These operations include intensive milling and mixing, sieving to control particle size distribution, and radiation sterilization using high-energy electrons or gamma irradiation to ensure microbiological stability without altering chemical composition. Such procedures are well documented in the literature and are routinely implemented by major CRM producers as matrix-specific adaptations within a common methodological framework. A step-wise comparison of production approaches applied to selected plant-based CRMs is presented in Table 1, using NIST SRM 1515 (Apple Leaves) and BARC-D3201 (Black Tea Powder) as representative examples.

Table 1 illustrates how a common production logic is implemented across different plant matrices while allowing for matrix-specific adaptations at individual processing stages, including sourcing strategy, pre-treatment scope, enrichment approach, and preservation method. Despite these differences, both materials fulfil identical certification requirements, including demonstrated homogeneity, stability, and satisfactory performance in interlaboratory comparisons. This comparison highlights that ISO compliance is achieved through structured quality assurance rather than through rigidly uniform production procedures.

The analytical characterization of plant-based CRMs is closely aligned with routine laboratory practice in food and environmental analysis. Pressure digestion methods, as described in European Committee for Standardization (2014), are commonly combined with inductively coupled plasma mass spectrometry (ICP-MS) for ultra-trace multi-element analysis or with inductively coupled plasma optical emission spectrometry for screening of major and selected trace elements. Mercury is frequently determined either within ICP-MS workflows or by cold vapor atomic absorption spectrometry, while graphite furnace atomic absorption spectrometry remains widely used for targeted determinations of elements such as cadmium and lead. X-ray fluorescence offers a non-destructive alternative for solid samples when higher limits of detection are acceptable. Across these techniques, matrix-dependent limits of quantification typically range from 10^{-4} mg kg^{-1} to 1 mg kg^{-1}, depending on the analytical method, sample mass, and dilution strategy.

Although several plant-based certified reference materials are commercially available, their diversity remains limited with respect to matrix type, analyte coverage, and concentration ranges. Selected examples commonly used for trace-element determinations are summarized in Table 2.

Table 2 shows that leafy matrices and cereal-based materials are relatively well represented, whereas fruit matrices remain largely absent. In particular, while strawberry leaves are available as a CRM for trace-element analysis, no certified reference material currently exists for strawberry fruit, nor for plant matrices enriched with toxic elements at concentrations aligned with food-safety legislation. Table 2 also highlights practical constraints faced by laboratories, including differences in minimum test portion, the availability of certified versus information values, and the relatively high cost of some materials, which may limit their routine application. Together, Tables 1 and 2 provide complementary perspectives on the current state of plant-based certified reference materials. Table 1 reflects the producer perspective,

Table 2. Selected plant-based CRMs commonly used for trace-element determinations (Supleco, 2019; LGC Standards, 2024b; BAM, 2023; LGC Standards, 2024a; European Commission & Joint Research Centre, 2026)

Producer	Code	Matrix	Number of elements (certified/information)	Minimal mass [g]	Mass/price
NIST	SRM 1515	Apple leaves	21/1	1–2	50 g/1400 EUR
NIST	SRM 1570a	Spinach leaves	18	0.15	60 g/1400 EUR
NIST	SRM 1573a	Tomato leaves	21/20	1–2	50 g/1300 EUR
JRC (BCR)	BCR-679	White cabbage	11/7	0.1–0.25	15 g/215 EUR
JRC (ERM)	ERM-CD281	Rye grass	14/8	0.2	10 g/200 EUR
LGC	LGC7162	Strawberry leaves	19/5	0.5–2	20 g/800 EUR
NMIJ (AIST)	CRM-7532	Rice flour	8/As compounds	0.5	20 g/170 EUR

demonstrating how ISO-aligned workflows are implemented to achieve homogeneity, stability, and traceability for complex plant matrices. Table 2 reflects the end-user perspective, revealing gaps in matrix coverage and practical limitations in the applicability of existing materials. These observations clearly indicate the need for new plant-based CRMs that better address analytical demands arising from food-safety legislation, environmental monitoring, and evolving regulatory requirements.

The work draws on the authors' established experience in the production of reference materials, encompassing the preparation and processing of candidate materials, followed by their involvement in certification activities and in the evaluation of measurement uncertainty budgets for complex matrices. Earlier studies addressed practical aspects of reference material production, such as matrix pre-treatment, assessment of within-unit and between-unit homogeneity, and participation in interlaboratory comparisons, as demonstrated for a complex biological matrix in the preparation of a human hair intercomparison material (Godlewska-Żyłkiewicz et al., 2002). Subsequent work included direct participation in international certification exercises within joint research projects, contributing to the characterization and certification of environmental reference materials, including the assignment of total element mass fractions in a soil certified reference material (Isleyen et al., 2024). In parallel, methodological studies focused on the evaluation of measurement uncertainty budgets, including explicit treatment of material-related contributions in trace and isotope ratio determinations (Karasiński et al., 2024). These activities were conducted within a broader metrological framework for chemical measurements, including the role of certified reference materials, as described in detail in the metrological literature (Bulska, 2018). This cumulative experience, spanning different matrix types and successive stages of the reference material life cycle, provided the methodological and metrological foundation for the design and implementation of the MultiBio CRM production strategy presented in this work.

Building on this established experience in the production and certification of reference materials, as well as in the evaluation of measurement uncertainty, the present study introduces a metrological strategy for the development of MultiBio CRM, a paired strawberry leaf and fruit certified reference material designed as a multi-parameter, multi-technique material. The term "multi" refers both to the assignment of certified values for multiple analytes, including trace elements, and to the intentional design of the material for application across a broad range of analytical measurement techniques. The proposed material is intended to support laboratories working with plant matrices using various analytical methods. The production process was carried out in accordance with ISO 17034:2016 and aligned with current international standards and guidance documents for reference materials, including ISO Guide 30:2015, ISO 33401:2024, ISO 33403:2024, and ISO 33405:2024. The workflow comprised matrix selection, controlled sampling, pre-treatment, drying or lyophilization, particle-size reduction, homogenization, stabilization, and final certification. Selected toxic elements, namely arsenic, cadmium, lead, and mercury, were incorporated at concentration levels consistent with ranges relevant to food-safety legislation and within analytical ranges suitable for both routine monitoring methods and high-sensitivity analytical techniques.

The present paper constitutes the first part of a series of studies dedicated to the development of the MultiBio certified reference materials. In this contribution, the primary focus is placed on the detailed description and critical evaluation of the production workflow, including matrix selection, controlled cultivation, pre-treatment, processing, stabilization, and ISO-aligned quality control. Subsequent publications will address the results of formal homogeneity studies, interlaboratory characterization, value assignment, and the final certification of the MultiBio CRM materials.

2 Experimental: Materials and Methods

The development of the MultiBio candidate materials followed a controlled production workflow designed to ensure suitability for routine laboratory applications and traceable assignment of property values. The workflow was implemented in accordance with ISO 17034:2016 and relevant guidance on the characterization, homogeneity, stability, and documentation of reference materials. Cultivation, processing, quality control, and documentation were carried out under controlled conditions at the University of Warsaw, ensuring traceable oversight throughout each stage of

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Table 3. ISO-aligned workflow and QC checkpoints for candidate for MultiBio CRM

Step	Purpose	Key parameters	QC checkpoint, acceptance
Matrix and lot selection	Ensure commutability and regulatory relevance	Plant part (fruit/leaf); defined harvest area and date; single bulk lot	Selection rationale documented; contamination survey negative; absence of atypical hotspots
Pre-treatment	Remove exogenous material; stabilise matrix	Minimal washing (if required); drying or lyophilisation to <3% (w/w) H_2O	Mass-loss curve documented; residual moisture meets specification; no thermal degradation
Particle-size reduction and control	Enable representative small test portions	Cryogenic grinding; preliminary mixing; optional sieving	No detectable coarse fraction ("coarse tail"); visually uniform powder
Bulk mixing/ homogenisation	Reduce spatial heterogeneity within batch	Tumbling or rotary mixing; time depending on matrix behaviour	Blend uniformity confirmed; no location-dependent trends
Targeted spiking	Adjust analyte levels to analytical and regulatory relevance	Aqueous standard solutions (As, Cd, Pb, Hg); controlled addition; re-drying and re-mixing	Agreement with target concentrations; no local enrichment effects
Bottling and packaging	Preserve homogeneity and chemical stability	Unit mass defined; bottling under inert atmosphere (argon purge); sealed containers	Consistent unit mass; container integrity verified; traceable labelling
Sterilisation	Ensure microbiological stability	Ionising radiation (e-beam or γ-irradiation); validated dose	No measurable chemical or physical alteration; sterility assured
Homogeneity studies	Demonstrate between- and within-bottle homogeneity	Random selection of bottles; replicate subsampling; ANOVA evaluation	Between-bottle variance ≤ analytical repeatability; fit for minimum sample mass
Stability studies	Confirm long-term material stability	Short- and long-term storage at defined temperatures; periodic analysis	No statistically significant trends over time within uncertainty
Certification and ILCs *)	Assign certified values and uncertainties	Interlaboratory comparisons; validated analytical methods; ISO 17034:2016 framework	Assigned values traceable to SI; uncertainties established; certification criteria fulfilled

Note: *) *This stage will be described in detail in Part II.*

production. The overall workflow and quality control checkpoints are summarized in Table 3.

This section describes the materials, key equipment, and stepwise procedures used to prepare the strawberry leaf and fruit candidate materials within the MultiBio CRM project. All devices used for mass and volume control during preparation and processing, including balances, pipettes, and dispensers, were calibrated and operated within defined acceptance limits. Laboratory environmental conditions were also monitored to help control sources of variability relevant to uncertainty evaluation and traceability documentation.

Key equipment used during material preparation and quality control included drying and lyophilization systems, agate mortars, stainless-steel cutting mills equipped with 40-mesh sieves, ball-milling and cryogenic grinding systems, rotating polyethylene drums for bulk mixing, calibrated balances, pipettes, and dispensers, closed-vessel acid-digestion systems with Teflon vessels, and ICP-MS instrumentation used for homogeneity screening (PerkinElmer NexION 300D).

2.1 Step 1: Matrix and Lot Selection–Commutability and Regulatory Relevance

In accordance with ISO Guide 30:2015 and ISO 17034:2016, the first step involved defining the intended use, measurand, and scope of the reference material, followed by deliberate selection of the plant matrix and production lot. Strawberries were selected due to their high relevance for food safety, environmental monitoring, and agricultural quality control. Poland ranks among the leading strawberry producers in Europe, placing tenth globally and second within the European Union in 2021 (ReportLinker, 2024), which underscores the analytical relevance of this matrix for European laboratories.

Strawberries are consumed both fresh and processed, while dried fruits and leaves are commonly used in fruit

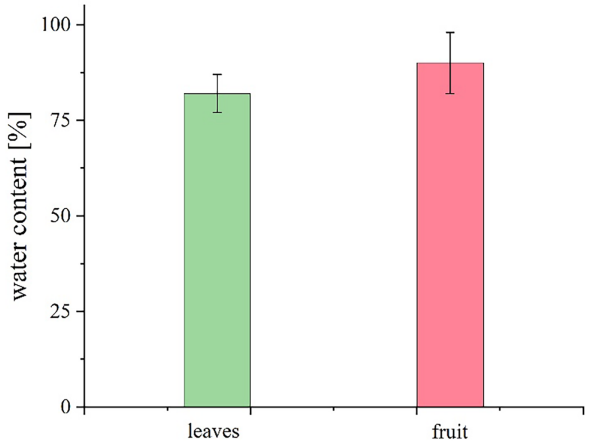

Figure 1. Preliminary water content of fresh strawberry leaves and fruit used to define the dehydration strategy

teas. From an analytical perspective, strawberries constitute a challenging matrix owing to their high water content and complex organic composition, which can strongly influence sample preparation, analyte extraction, and measurement repeatability. The water content of fresh strawberry fruit reaches approximately 90%, while that of leaves is approximately 80% (Figure 1). Such characteristics necessitate careful matrix selection and processing, as emphasized in ISO 33405:2024 for biological reference materials.

Both fruits and leaves were selected to represent analytically distinct plant tissues commonly encountered in routine laboratory practice. Plants were cultivated under controlled laboratory conditions to minimize biological variability and to allow full documentation of cultivation parameters, as required by ISO 17034:2016 for control of input materials. A single bulk lot was defined for each matrix, and harvest date, cultivation conditions, and batch identity were recorded. A preliminary contamination survey was carried out to exclude the presence of atypical contamination hotspots and to confirm the suitability of the plant material for further processing. The survey was based on the analysis of representative subsamples collected from different plants and cultivation locations, focusing on elements indicative of potential environmental contamination. Atypical contamination was defined as localized, non-representative enrichment of selected analytes exceeding the expected biological variability for the given matrix. Evaluation of the results showed no evidence of such enrichment, confirming the suitability of the material for reference material production. The botanical identity of the plant material was verified and documented prior to processing to ensure matrix authenticity and commutability, in accordance with recommendations for plant-based reference materials.

2.2 Step 2: Sample Collection, Storage, and Pre-Treatment–Removal of Exogenous Material and Moisture Stabilisation

Sample collection and handling procedures were developed by adapting established protocols reported for plant-based reference materials (ReportLinker, 2024; López-Romero et al., 2005) and aligning them with ISO 33405:2024 guidance, which emphasizes minimization of contamination and preservation of matrix integrity. Strawberry fruits and leaves were collected separately and placed into dedicated, clearly labelled containers to prevent cross-contamination and to maintain traceability throughout processing. Fruits were harvested successively at full technological maturity to reflect consumer-relevant exposure profiles, whereas leaves were collected as a single batch to minimize within-lot biological variability and to strengthen batch definition under ISO 17034:2016 control of input materials. Where required, samples were briefly washed to remove exogenous particulates using a protocol described in the literature and commonly applied in CRM preparation, consisting of tap water with gentle mechanical action, a short dip in 0.1 mol L^{-1} HCl, and five rinses with deionized water, with the total washing time kept below one minute (López-Romero et al., 2005). This specific sequence and duration were selected to balance effective removal of surface contamination with avoidance of leaching or alteration of endogenous elemental composition, in accordance with ISO 33405:2024. Given the intrinsically high water content of strawberry matrices (Figure 1), prompt moisture reduction was identified as a critical stabilization step.

Drying of leaves (Dybczyński et al., 1998) and lyophilisation of fruits (Kiełbasa et al., 2016) were selected based on literature evidence demonstrating their effectiveness in reducing moisture while preserving matrix composition. Residual moisture was reduced below 3% (w/w), a threshold reported to enable room-temperature storage of biological materials (Venelinov & Quevauviller, 2003). Moisture loss was monitored gravimetrically, and mass-loss curves were documented as part of process control.

Drying conditions followed established literature precedents; for example, tobacco leaves have been air-dried and subsequently dried at 40°C (Dybczyński et al., 1998). Moisture-uptake experiments were conducted to verify literature observations under the present laboratory conditions. As shown in Figure 2, strawberry fruits absorbed moisture more rapidly and to a greater extent than leaves upon exposure to laboratory air, reaching approximately 20% and 15% moisture content, respectively, after 24 h, with the steepest increase occurring within the first 0.5 h–5 h. These findings directly indicated the necessity of minimizing exposure time between drying or lyophilisation and packaging. Consequently, materials were promptly transferred into tightly sealed glass containers or polyethylene bags, which were labelled with collection date, drying conditions, and sample mass. To support traceability and uncertainty control, all transfers and weighing operations during pre-treatment were recorded in production logs, including sample masses before and after moisture reduction, container identifiers, and processing dates, consistent with documentation expectations for reference material production.

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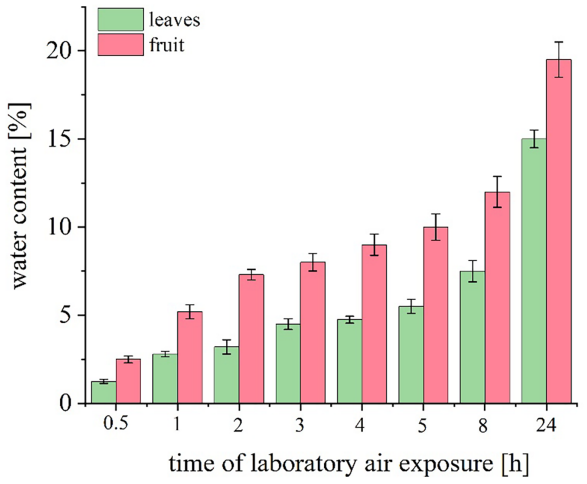

Figure 2. Moisture uptake of dried strawberry materials during exposure to laboratory air; preliminary process-screening data used to define handling times. Note: Figures 1 and 2 present preliminary process-screening measurements used to establish dehydration and handling conditions. These data are provided to justify process-selection decisions rather than to report certified values; nevertheless, the dispersion of the measurements, expressed as standard deviation (n = 3), is included.

2.3 Step 3: Particle-Size Reduction and Control–Preparation for Representative Subsampling

Following pre-treatment and stabilization, defined as moisture reduction to below 3% (w/w) and protection of the material against moisture uptake and biological degradation through controlled handling and sealed storage, the plant material was subjected to controlled particle-size reduction to enable representative subsampling at analytically relevant test portions. ISO 33405:2024 identifies particle size as a critical parameter influencing achievable homogeneity and the minimum test portion that can be declared for a reference material. For this reason, particle-size targets reported in the literature for plant-based CRMs were explicitly adopted as design criteria for the MultiBio CRM materials.

Grinding was carried out using a defined, multi-stage comminution procedure. Pre-dried and stabilized plant material was first manually fragmented in agate mortars to reduce bulk particle size and to ensure controlled handling of small batches. Final particle-size reduction was then performed using mechanical milling systems, specifically stainless-steel cutting mills equipped with 40-mesh sieves, followed by ball milling to achieve fine and uniform powders. For matrices prone to agglomeration or thermal sensitivity, cryogenic grinding was applied as the final comminution step, in accordance with literature recommendations, in order to minimize heat generation and to prevent thermal degradation, volatilization, or redistribution of analytes within the matrix.

Target particle-size ranges were selected based on established practice. According to Dybczyński et al. (2004) particle sizes of ≤67 µM are typically applied for tea leaves and herbal materials, while ≤80 µM is used for tobacco leaves. A 80 µM threshold was therefore adopted in this study as practical benchmarks. Ground material was passed through appropriate sieves to achieve a narrow particle-size distribution and to remove oversized particles. Fruit matrices required additional grinding cycles due to their soft tissue structure and tendency to form agglomerates.

This step was considered complete when no coarse material remained after sieving and the powder appeared visually uniform throughout the batch. To avoid cross-contamination during grinding, all tools and milling chambers were cleaned between processing steps according to a defined procedure. This included washing with distilled water, rinsing with alcohol to remove residual organic material, and drying completely before reuse. Cleaning records documented the procedure, acceptance criteria, and verification of cleaning effectiveness by visual inspection and, where applicable, blank measurements. All cleaning activities and verification results were retained as part of the production documentation.

2.4 Step 4: Bulk Mixing and Homogenization–Reduction of Spatial Heterogeneity

Bulk homogenization was carried out to minimize spatial heterogeneity within the batch and to ensure uniform distribution of analytes, which is a prerequisite for the assignment of batch-level certified values under ISO 17034:2016. Mixing strategies reported in the literature for plant-based reference materials were adopted, with thorough mixing performed in rotating polyethylene drums.

Mixing durations were selected based on literature experience and preliminary tests, typically around 16 h, with longer durations applied where slower equilibration was observed (Dybczyński et al., 1998). For matrices prone to aggregation, particularly finely ground fruit materials, auxiliary dispersion techniques such as ultrasonic mixing were applied selectively, as described in previous CRM studies (Dybczyński et al., 1998). Homogeneity was assessed using statistically designed experiments, with analytical data obtained by inductively coupled plasma mass spectrometry (López-Romero et al., 2005; Dybczyński et al., 1998; 2004). In the case discussed, a PerkinElmer NexION 300D was used, operated in wet plasma mode with a cyclonic spray chamber and a Meinhard nebulizer at a sample uptake rate of 100 µL min^{-1}. The interpretation of analytical repeatability and its role in evaluating measurement variability was based on the definitions and statistical principles for repeatability and reproducibility provided in ISO 5725-2:2025 (2025). Statistical evaluation using analysis of variance (ANOVA) and Levene's test followed the principles outlined in ISO 33405:2024. Homogenization was considered satisfactory when between-sample variability did not exceed analytical repeatability and no location-dependent trends were observed.

Where preliminary screening indicated insufficient distribution of enriched analytes (As = 23%, Hg = 46%, Pb = 25%, Cd = 30%, expressed as RSD) of enriched analytes, extended mechanical mixing was applied for additional periods, and

the sequence of corrective homogenisation steps was documented to support process transparency and subsequent interpretation of homogeneity results.

2.5 Step 5: Targeted Spiking–Adjustment of Analyte Content

Where native concentrations of selected analytes were below analytically or legislatively relevant levels, targeted spiking was applied to adjust concentration ranges while preserving matrix integrity. This approach is consistent with ISO 33405:2024 guidance on formulation of biological reference materials and with other recommendations for producing fit-for-purpose materials.

Aqueous standard solutions of arsenic, cadmium, lead, and mercury were added under strictly controlled conditions to the homogenized bulk material. The spiking procedure was designed to ensure quantitative transfer and uniform distribution of analytes throughout the batch. The calculated volume of the single-element standard solution was added to the material after preliminary cryogenic grinding. The total volume of the standard solution was approximately 1 mL (depending on the element) and, in all cases, it was added in aliquots of approximately 20 µL–30 µL to ensure the most uniform possible distribution within the material. Following spiking, the material was subjected to additional mixing to restore physical uniformity.

Spiking solutions were added incrementally in small aliquots with intermediate mixing to minimize local enrichment effects. The selection of spiking levels was guided by food-safety relevance and laboratory measurement ranges, and all added volumes and calculated mass fractions were recorded to support traceability and mass-balance control. For example, for the leaf material, the target mass fractions of the enriched elements in the final material were: Pb = 1.0 mg kg^{-1}, Cd = 0.4 mg kg^{-1}, As = 0.5 mg kg^{-1}, and Hg = 0.2 mg kg^{-1}.

2.6 Step 6: Bottling and Packaging–Preservation of Material Integrity

Bottling and packaging were performed in accordance with ISO 17034:2016 and ISO 33401:2024 requirements for control of packaging operations and documentation. The homogenized bulk material was subdivided into defined unit masses selected (12 g for leaves and 10 g for fruits) to balance homogeneity requirements, minimum test portion constraints, and practical laboratory use.

Bottling was carried out under an inert atmosphere (argon purge) to minimize oxidation and moisture uptake, particularly important for finely powdered botanical matrices. Containers were hermetically sealed and labelled with unique identifiers to ensure valid identity throughout storage and distribution. Unit mass consistency, container integrity, and label accuracy were verified prior to release of the batch. The filling sequence of individual units was recorded to enable subsequent evaluation of potential filling-order trends during homogeneity assessment, consistent with ISO 33405:2024 recommendations for study design.

2.7 Step 7: Sterilization–Microbiological Stability

ISO 33405:2024 highlights microbiological stability as a key requirement for biological reference materials. Sterilization was therefore performed using ionizing radiation, such as electron-beam or gamma irradiation, with dose ranges selected based on extensive literature evidence (Dybczyński et al., 2004; Wise et al., 2025; Kawamoto et al., 2019; Martínez-Francés et al., 2023). Doses of 27 kGy–30 kGy or, for selected matrices, 5 kGy–10 kGy have been reported to be effective in preventing microbial degradation without inducing measurable chemical or physical changes (Dybczyński et al., 1998; 2004; Wise et al., 2025; Kawamoto et al., 2019) and these values were adopted in the present study. Electron beam sterilization was applied with an irradiation dose of 28 kGy.

The absence of measurable changes after sterilization was verified by comparing pre- and post-irradiation moisture content, visual appearance, and selected elemental screening results. No statistically significant differences were observed within method precision. These observations confirmed that the literature-supported sterilization conditions were suitable for the MultiBio CRM materials and did not adversely affect their relevant physical or analytical properties.

Sterilization was performed by a specialized external facility, and the irradiation documentation was retained as part of the production records to support traceability and conformity assessment in accordance with ISO 17034:2016.

2.8 Step 8: Homogeneity Studies

Between-bottle and within-bottle homogeneity of the MultiBio candidate for certified reference material was evaluated in accordance with the requirements of ISO 33405:2024. The objective of this study was to demonstrate that the prepared lot is sufficiently uniform at the defined minimum test portion and to quantify the contribution of potential inhomogeneity to the overall uncertainty budget. Randomly selected units were analyzed in replicate using validated analytical methods appropriate for the target analytes and the plant matrix, and ANOVA statistical evaluation was applied to distinguish material-related variability from analytical repeatability.

For the fruit-based candidate material, a structured homogeneity testing design was implemented. The batch comprised 65 individual units, each containing approximately 15 g of material, packaged in 25 mL glass containers under an argon atmosphere. All units were sequentially numbered during filling to allow evaluation of potential trends related to packaging order. The full population of units was divided into seven subsets reflecting the filling sequence, with subsets 1 and 4 containing 10 units each and subsets 2, 3, 5, 6, and 7 containing 9 units each. From each subset, one unit was randomly selected for homogeneity testing. From each selected unit, four independent test portions were prepared at the defined minimum test mass, yielding a total of 28 analytical subsamples.

All analytical measurements were performed under repeatability conditions, meaning that analyses were carried out on the same day, by the same analyst, using the same

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instrumentation and reagents. This approach ensured that analytical variability was minimized and that observed dispersion could be attributed primarily to material-related effects. To control for potential instrumental drift and to detect possible effects related to the filling order, the 28 subsamples were grouped into four analytical series, each containing one subsample from each selected unit. The order of units within each series was deliberately permuted. The unit sequences applied were 3, 7, 4, 2, 5, 1, 6 in Series 1; 7, 6, 5, 4, 3, 2, 1 in Series 2; 4, 6, 1, 7, 3, 5, 2 in Series 3; and 2, 6, 3, 7, 4, 1, 5 in Series 4. This balanced design enabled the identification of systematic trends associated with measurement order, instrumental drift, or packaging sequence.

Sample preparation for homogeneity testing involved closed-vessel acid digestion to ensure complete dissolution of the plant samples. Approximately 0.3 g of material was weighed into Teflon digestion vessels using calibrated balances. Concentrated nitric acid (5 mL) and hydrogen peroxide (1 mL) were added, and digestion was carried out using a temperature-controlled program reaching 230°C (30 minutes ramping) with a defined holding period (15 minutes). After cooling, the digests were diluted gravimetrically to a final mass of approximately 50 g. Elemental determinations were performed by inductively coupled plasma mass spectrometry using external calibration combined with internal standard correction.

Calibration for arsenic, cadmium, and lead was performed using mixed calibration solutions prepared from single-element stock standards by gravimetric dilution in 1% nitric acid. Target calibration concentrations were 3.0 µg per kilogram for arsenic, 2.4 µg per kilogram for cadmium, and 6.0 µg per kilogram for lead. Any potential systematic error arising from volumetric preparation was considered negligible for the purposes of the homogeneity study, as relative differences between subsamples were evaluated rather than absolute values. An internal standard solution containing germanium, indium, and thallium was prepared in 1 percent nitric acid, with target concentrations of approximately 30 µg/L to 50 µg/L for germanium and 10 µg/L to 20 µg/L for indium and thallium. Mercury calibration was performed using a nitric acid solution stabilized with gold to minimize adsorption and volatilization losses, and internal standard correction was applied in the same manner as for the other elements. For the rest of elements, calibration standards were prepared from a multi-element stock solution using a three-point calibration curve corrected for blank signals, with recommended calibration levels of 5 µg/kg, 15 µg/kg, and 25 µg/kg. Internal standard correction was applied consistently, with arsenic signals corrected using germanium, cadmium signals using indium, and mercury and lead signals using thallium. The recommended isotopes monitored during the homogeneity study included ^{75}As, ^{74}Ge, ^{111}Cd, ^{115}In, ^{202}Hg, ^{205}Tl, and ^{208}Pb, with the option to monitor a single lead isotope for homogeneity evaluation.

Prior to variance analysis, the analytical datasets were evaluated for normality, and the presence of gross outliers was assessed using Grubbs' test. Any exclusions were justified and documented in accordance with ISO guidance. Potential instrumental drift or filling-order effects were evaluated by linear regression of elemental mass fraction versus subset number. The slope coefficient and its standard deviation were calculated, and the statistical significance of the slope was assessed using a two-tailed t test at the 95 percent confidence level. Ordinary least squares provided the slope coefficient b and its standard deviation Sb. A two-tailed t-test assessed the significance of the slope using the statistic:

$$t = \frac{b}{Sb}$$

b–slope coefficient

Sb–standard deviation of the slope coefficient

Then the obtained t-value was compared with the t_{critic} value read from the Student's t-distribution table (two-sided test variant) for 5 degrees of freedom and a confidence level of 95% (t_{critc} = 2.571).

The absence of statistically significant slopes confirmed that no systematic trends related to analytical sequence or packaging order were present.

Quantification of material homogeneity was performed using a two-factor analysis of variance without replication. The analysis provided mean squares representing between-unit variability and within-unit variability. These values were used to estimate the corresponding variance components, with the between-unit variance calculated from the difference between the between-unit and within-unit mean squares divided by the number of replicate subsamples per unit, and negative estimates set to zero in accordance with established metrological practice. The within-unit variance was taken directly from the within-unit mean square. The combined standard uncertainty associated with material inhomogeneity was calculated as the square root of the sum of the between-unit and within-unit variance components. This contribution was incorporated into the overall uncertainty budget for value assignment and certification, ensuring that potential spatial or packaging-related variability was explicitly captured and documented in full compliance with ISO 33405:2024.

2.9 Step 9: Stability Studies

Stability studies were designed and evaluated following ISO 33405:2024 requirements. Both short-term and long-term stability were assessed under defined storage conditions, with samples analysed at predetermined intervals over extended periods.

Regression analysis was applied to detect potential temporal trends in analyte concentrations. Stability was confirmed when no statistically significant changes were observed within the stated measurement uncertainty (ISO/IEC 17025:2017, 2017; Karasiński et al., 2024; ISO 5725-2:2025, 2025; Wise et al., 2025; Kawamoto et al., 2019). These studies provided the basis for defining recommended storage conditions and shelf life.

Where appropriate, an isochronous design was applied, whereby units stored for different durations were analyzed within the same analytical sequence at the end of the study,

minimizing the influence of day-to-day analytical variation on trend detection. This design is consistent with ISO 33405:2024, which defines an isochronous stability study as one in which units exposed to different storage times or conditions are measured within a short analytical period to reduce the influence of day-to-day analytical variation (ISO 33405:2024, 2024).

2.10 Step 10: Certification and Interlaboratory Comparisons

Final certification of the MultiBio CRM reference materials was carried out in full compliance with the requirements of ISO 17034:2016, ISO 33401:2024, and ISO 33405:2024, as well as with the recommendations provided in the ILAC Guidelines (International Laboratory Accreditation Cooperation, 2005) for the selection and use of reference materials. Certification was based on results obtained from interlaboratory comparisons involving independent and competent laboratories applying validated analytical methods, including inductively coupled plasma mass spectrometry (ICP-MS), instrumental neutron activation analysis (INAA), X-ray Fluorescence (XRF) and flame/electrothermal atomic absorption spectrometry (F/ET AAS). The participation of multiple laboratories and the use of complementary analytical techniques were essential to ensure the robustness of the assigned values and to minimize method-dependent bias, in accordance with the principles outlined in ISO 33405:2024.

All reported interlaboratory results underwent comprehensive statistical evaluation, including the assessment of data consistency, identification and treatment of outliers, and estimation of between-laboratory variability. Certified values were assigned using appropriate statistical models, such as weighted means where justified, and associated measurement uncertainties were established by combining contributions from characterization, homogeneity, and stability studies. The uncertainty evaluation followed the framework defined in ISO 33405:2024, ensuring that all relevant sources of variability were considered and transparently documented.

Preparation of the certified reference material certificate followed the requirements specified in ISO 33401:2024, which define both the mandatory content and the structure of reference material documentation. The certificate includes a unique identification of the certified reference material and its producer, a detailed description of the matrix and intended use, the certified property values with their expanded uncertainties and coverage factors, and a clear statement of metrological traceability to the International System of Units. In addition, the certificate provides a description of the analytical methods used for characterization and uncertainty estimation, summaries of homogeneity and stability assessments, recommended storage and handling conditions, the minimum test portion, and the defined period of validity of the certification.

All information included in the certificate and accompanying documentation was critically reviewed to ensure internal consistency, technical clarity, and usability by end users, in line with ISO 33401:2024 requirements. The documentation was prepared to enable laboratories to directly apply the MultiBio CRM certified reference materials for method validation, internal quality control, estimation of measurement uncertainty, and the formal demonstration of metrological traceability in accordance with ISO/IEC 17025:2017 and ISO 15189:2022. Completion of this stage marked the formal release of the MultiBio certified reference materials for routine laboratory use.

3 Results and Discussion

3.1 Metrological Framework and Normative Basis for CRM Production

The acceptance and practical usability of a plant-based certified reference material depend directly on how it is produced. Commutability, homogeneity at the minimum test portion, stability over the period of validity, and a well-supported uncertainty statement with complete documentation are essential requirements for its use in analytical practice. These requirements are defined in international standards governing reference material production, characterization, value assignment, and documentation. From the user's perspective, compliance with these requirements determines whether a CRM can be validly used for method validation, internal quality control, uncertainty estimation, and demonstration of metrological traceability in accordance with ISO/IEC 17025:2017 and ISO 15189:2022. In this work, the production and evaluation of the MultiBio materials were aligned with ISO 17034:2016 and relevant international guidance. The results should therefore be understood not only as analytical data, but also as evidence that the material is fit for routine and regulated laboratory use.

In the broader context of food safety and functional food development, the role of certified reference materials extends beyond analytical quality assurance and becomes a prerequisite for the verification of elemental composition, nutritional claims, and regulatory compliance. This requirement is particularly critical for strategies involving controlled enrichment of crops with essential elements, where reliable and traceable measurements are essential to ensure consumer safety and environmental sustainability. These challenges are explicitly addressed within the framework of the PATHFOOD project (Creating a Functional and Sustainable Food Chain: A Path to Climate Neutrality and Improved Health in the EU), which integrates sustainable crop cultivation, robust traceability systems, and advanced analytical control as key pillars of future European food systems. In this context, the availability of well-characterised, matrix-matched plant-based certified reference materials is indispensable for the validation of enrichment strategies, verification of regulatory limits, and harmonisation of measurement results across laboratories participating in such initiatives.

3.2 Demonstration of Metrological Traceability and Documentation of Matrix Provenance in Plant-Based Certified Reference Materials

The development of the MultiBio materials shows that metrological traceability in plant-based CRMs depends on

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both analytical rigor and careful control of matrix history. Traceability is established through an unbroken chain of comparisons linking assigned values to the SI, with all associated uncertainties stated. In practice, however, this chain remains meaningful only when the material origin, cultivation, harvesting, and processing are sufficiently controlled to preserve commutability, homogeneity, and stability.

For this reason, the MultiBio workflow placed strong emphasis on early control of cultivation and handling. Variations in plant maturity, environmental exposure, or post-harvest treatment can affect both composition and physical behavior during drying, milling, and mixing. Once introduced into the bulk material, such effects are difficult and costly to correct.

3.3 Material Homogeneity as a Critical Control Parameter and Management of Non-Conformities

Material homogeneity is one of the most critical control parameters in the production of powdered plant-based certified reference materials, as it determines whether a single certified value can be validly applied at the declared minimum test portion. Owing to the intrinsic heterogeneity of plant tissues and the combined effects of drying, milling, spiking, mixing, and bottling, homogeneity cannot be assumed but must be demonstrated experimentally and included in the uncertainty evaluation in accordance with ISO-aligned guidance for reference material characterization. The results obtained during the MultiBio production illustrate how homogeneity evolved during processing and how deviations from predefined criteria were addressed within the producer's quality framework. Similar approaches have been reported for food and environmental CRMs, where the minimum sample mass and the uncertainty contribution associated with heterogeneity were established explicitly during material development (Polkowska-Motrenko et al., 2022).

Following spiking and initial comminution, preliminary homogeneity screening showed elevated relative standard deviation (RSD) values for the fortified elements: 22.7% for arsenic, 35.7% for cadmium, 45.6% for mercury, and 25.2% for lead. At the same time, the corresponding mean mass fractions were already consistent with the predefined target concentrations. This combination of acceptable mean values and excessive dispersion indicates that the spiking procedure achieved the intended average analyte levels, but not yet a sufficiently uniform spatial distribution within the bulk material. Such behavior is commonly observed in fortified plant matrices and may result from localized wetting during spiking, adsorption effects, agglomeration, or incomplete equilibration after re-drying.

Table 4 summarizes the evolution of heterogeneity, expressed as RSD, for the fortified elements at successive stages of process optimization. Additional mechanical mixing reduced dispersion for all fortified elements relative to the initial state. A further improvement was obtained when the declared minimum test portion was increased from 250 mg to 500 mg, indicating that residual microscale heterogeneity could be partly averaged by larger test portions. Mercury consistently showed the highest RSD values, confirming its more challenging behavior in fortified plant matrices. This observation is consistent with previous CRM studies showing that within-unit homogeneity is closely related to the minimum sample intake and that sample sizes equal to or above this threshold support the validity of the certified value within its stated uncertainty (Grimalt et al., 2015).

The corrective action adopted in the MultiBio workflow was extended mechanical homogenization rather than post hoc adjustment of analytical procedures or assigned values. This approach is consistent with the ISO principle that insufficient material performance should be addressed primarily at the production stage. Each material was mechanically mixed for approximately two weeks, for 8 h per day. After extended mixing, the RSD values decreased to 16.0% for arsenic, 7.7% for cadmium, 21.1% for mercury, and 15.0% for lead. When the minimum test portion was increased from 250 mg to 500 mg, the corresponding RSD values decreased further to 8.6%, 7.7%, 20.0%, and 12.4%, respectively.

These results show that the initial lack of satisfactory homogeneity was predominantly material-related and that residual microscale heterogeneity could be reduced by improved equilibration and, where necessary, by increasing the declared minimum test portion. Elements not subject to spiking showed RSD values below 5% after cryogenic milling, indicating that the main homogeneity challenge was associated with fortification rather than with comminution itself. In practice, when homogeneity remains insufficient after re-mixing, the producer may need to increase the declared minimum test portion or include the residual heterogeneity contribution in the uncertainty budget, provided that the resulting expanded uncertainty remains compatible with the intended use of the material. In addition, homogeneity assessment should remain sensitive to possible filling-order effects, as segregation during bottling may compromise batch-wide certification even when the bulk blend appears acceptable.

3.4 Stability Performance, Packaging Strategy, and Interpretation of Stability Indicators

Stability performance is a key determinant of whether a CRM remains usable over time and under realistic transport and storage conditions. For plant-based materials, stability is strongly influenced by moisture, oxygen, light, and microbiological activity, making packaging strategy an integral component of the production process rather than a secondary consideration.

In the MultiBio production, inert-atmosphere packaging and sterilization were implemented to mitigate degradation pathways that would otherwise limit shelf life or compromise commutability. From the user perspective, these measures directly affect whether the CRM can be used without imposing impractical storage conditions or excessive handling constraints. ISO-aligned stability studies and transparent reporting allow laboratories to assess whether a material remains suitable for its intended applications over the declared validity period. When stability studies indicate potential drift, ISO guidance provides a clear framework for

Table 4. Evolution of material heterogeneity for fortified elements (As, Cd, Pb, Hg) expressed as relative standard deviation (RSD, %) at successive optimisation stages: initial state after spiking, after extended mechanical homogenisation, and after increasing the declared minimum test portion from 250 mg to 500 mg.

Element	Heterogeneity [RSD]		
	Initial	After additional mechanical mixing	After increasing the minimum test portion from 250 mg to 500 mg
As	22.7	16.0	8.6
Cd	35.7	7.7	7.7
Pb	25.2	15.0	12.4
Hg	45.6	21.1	20.0

Note. RSD values were calculated from replicate subsamples analyzed at the stated minimum test portion. The lower RSD values obtained at 500 mg reflect improved averaging of residual microscale heterogeneity rather than a change in mean concentration.

interpretation and corrective action. Producers must distinguish analytical effects from genuine material changes and, where necessary, adjust storage recommendations or shorten the period of validity. Overstating stability undermines user confidence and can lead to incorrect application in regulated contexts, whereas realistic claims supported by evidence enhance the credibility and utility of the CRM.

3.5 Measurement Uncertainty as an Integral Component of Certification and Fitness for Purpose

The uncertainty associated with a certified value summarizes the variability remaining after production control and characterization. For users, it defines the applicability of the CRM in conformity assessment, trend analysis, and regulatory decision-making. For producers, it indicates how effectively critical sources of variability have been controlled.

The MultiBio results show that, particularly for fortified plant matrices, material heterogeneity may dominate the uncertainty budget over analytical performance. This demonstrates that ISO-aligned uncertainty evaluation is not merely a reporting requirement, but a practical tool linking production quality to end-user applicability. The homogeneity contribution should therefore be explicitly quantified and included in the uncertainty model.

Applied iteratively during development, uncertainty budgeting can identify process limitations at an early stage and support decisions on further mixing, adjustment of the minimum test portion, or process redesign. This helps avoid failure at certification and improves decision-making within the production system.

3.6 Documentation, Certification Readiness, and Regulatory Usability of the CRM

The practical usability of a certified reference material is ultimately determined not only by its physicochemical properties, but also by the completeness, clarity, and internal consistency of its certificate and accompanying documentation. For laboratories operating within accreditation and regulatory frameworks, the certificate represents the formal and auditable basis for the application of the material in method validation, internal quality control, estimation of measurement uncertainty, and the demonstration of metrological traceability. Consequently, even a technically well-produced material may be rendered unsuitable for regulated use if its documentation does not meet normative expectations.

The results obtained in the MultiBio study demonstrate that documentation must provide a coherent narrative linking production strategy, material processing, characterization data, homogeneity and stability assessment, and uncertainty evaluation. Each certified value must be supported by transparent evidence showing how it was assigned, how its uncertainty was derived, and under which conditions it remains valid. Ambiguities in the definition of the measurand, minimum test portion, storage requirements, or period of validity directly undermine the ability of users to apply the material in a compliant manner.

3.7 Practical Implications for Plant-Based CRM Production and Corrective Decision Pathways

The combined MultiBio results and current normative requirements highlight a small set of critical control factors: moisture management, particle-size control, spiking strategy, and statistically sound study design. Weaknesses at any of these stages can directly affect homogeneity, stability, uncertainty, and ultimately material usability. ISO-aligned production provides a structured framework for identifying deviations, implementing corrective actions, and documenting outcomes. In this context, the key practical message is that plant-based CRM production is successful only when processing, metrological evaluation, and documentation are managed as one integrated system. This integration is essential to produce materials that are scientifically robust and fit for routine, accredited, and regulatory use.

4 Conclusions

This work establishes a practical and ISO-aligned metrological framework for the development of plant-based certified reference materials and demonstrates its application through the preparation of a paired strawberry leaf and fruit material within the MultiBio project. The study shows that controlled cultivation, matrix-appropriate dehydration, fine comminution, extended homogenization, inert-atmosphere

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packaging, and structured assessment of homogeneity and stability can be integrated into a traceable production strategy for complex plant matrices. This approach is particularly relevant for fruit-based materials, for which suitable CRMs remain limited.

The implemented workflow was designed to address the main challenges associated with biological matrices, including intrinsic heterogeneity, high moisture content, and sensitivity to processing and environmental conditions. The results demonstrate that reliable CRM production depends not only on analytical performance, but also on effective control of the production process and on the integration of metrological evaluation with material preparation. The application of ISO 17034:2016, together with current supporting ISO documents, provided a clear framework for producer competence, process control, decision-making, and documentation. The developed materials respond to a practical need for traceable calibration, method validation, and quality control in the determination of elemental contaminants in plant-derived food matrices, with particular relevance to Polish and European monitoring laboratories.

The present paper focuses on production strategy and process control as the methodological foundation of the MultiBio CRM. Part II of this study will present the quantitative assessment of homogeneity and stability, together with value assignment, uncertainty evaluation, and final certification of the developed materials. Future work will also extend analyte coverage, strengthen the interlaboratory basis for certified values, and expand the MultiBio portfolio to additional plant matrices relevant to food, agricultural, and environmental analysis.

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Availability of Data and Materials

The authors declare that should any raw data files be needed about the further data of the study, they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Ethics Approval

This study did not involve human participants, human biological material, personal data, or animals; therefore, ethical approval was not required under applicable institutional or national regulations. The research was conducted exclusively on plant material cultivated and processed under controlled laboratory conditions. Human involvement was limited to routine scientific and technical activities performed by the authors, including plant cultivation, sample preparation, instrumental analysis, and data evaluation, and did not constitute research involving human subjects. All experimental procedures were carried out in accordance with

applicable laboratory safety, environmental, and institutional requirements.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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