MINISTERUL EDUCAȚIEI ȘI CERCETĂRII
Universitatea POLITEHNICA din București
Școala Doctorală de
Inginerie Industrială și Robotică

M.Sc.Eng. Ovidiu Mărculescu

DOCTORAL THESIS
(SUMMARY)

OPTIMIZATION OF QUALITY MANAGEMENT IN AGRO-FOOD PRODUCT TESTING

Coordinator:
Prof.Univ.Habil.Dr.Eng.Ec.Mat. Augustin SEMENESCU

- 2024 -

Table of Contents

Introduction

In the current context of the agro-food industry, product testing has become essential due to the increase in negative phenomena such as contamination, falsification and product deterioration. In addition to these challenges, stringent regulations imposed by regulatory authorities aim to combat these harmful activities and ensure quality and food safety standards for consumers. These measures are necessary to maintain public health standards and consumer confidence in the integrity of the food products available on the market.

The continuous evolution of laboratory tests is essential to meet the increasingly stringent requirements of the agro-food industry and to facilitate the accurate and rapid detection of contaminants, pesticide residues, additivesand other hazardous chemicals. As the complexity and volume of tests conducted in testing and analytical laboratories increase, it is fundamental to demonstrate and maintain confidence in the obtained results.

In this context, reference materials play a crucial role, ensuring the proper calibration of measuring equipment and the validation of analytical methodologies. Accurate and reliable reference materials enable laboratories to perform comparable and reproducible tests, thus guaranteeing the accuracy and reliability of the results.

Reference materials represent fundamental pillars in the testing process of agro-food products and in quality management systems. These materials, defined as certified substances or materials that serve as reference points for comparing and evaluating the quality and performance of analyses, play a crucial role in ensuring the precision and reliability of food testing results.

Developing reference materials requires the establishment of a quality management system in accordance with ISO 17034, a complex and rigorous process but essential for the development of high-quality reference materials. ISO 17034 defines the necessary requirements to demonstrate the capability and competence of reference material producers, thus ensuring that these materials are suitable for use in instrument calibration, analytical method validation and quality control in laboratories.

Implementing this standard involves documenting and monitoring production processes, conducting rigorous checks and maintaining traceability of reference materials to national and international standards. Adhering to ISO 17034 is crucial to guarantee the accuracy, reliability and reproducibility of analytical results, thereby strengthening confidence in the produced reference materials.

In an environment characterized by rapid technological advancement and an accelerated pace of innovation in the food industry, the development and use of reference materials become increasingly necessary. This technological advancement impacts the diversification of food products and production processes, requiring the continuous adaptation of testing and certification methods to ensure their quality and safety.

Chapter 1. Introduction to Quality Management in the Food Industry

Quality management in the food industry is vital for ensuring product compliance with safety standards and meeting consumer expectations. The implementation of quality control measures has significantly evolved, involving advanced testing and precise measurements throughout the entire production process to guarantee product quality consistency. Regulatory legislation, such as the Food Safety Modernization Act (FSMA), has promoted the adoption of rigorous quality management systems, contributing to the minimization of food risks and contamination.

The integration of Hazard Analysis Critical Control Point (HACCP) principles into modern quality management systems has become standard practice, ensuring the management of potential risks at each production stage. This approach is supported by lean manufacturing techniques, which focus on reducing waste and improving operational efficiency while maintaining high-quality standards.

Major incidents regarding food product falsification have led to the development of stringent regulations to prevent food fraud, such as the European Parliament's 2013 resolution. The Parliament urged the European Commission to address food fraud rigorously and adopt measures to prevent and combat it.

In this context, the European Strategy Forum on Research Infrastructures (ESFRI) plays an essential role in policy development regarding research infrastructure in Europe, allocating substantial funds for the development of research projects in the field of food safety.

Regulations on the quality testing of food products represent essential pillars for ensuring the integrity and safety of food consumed globally. These regulations are established and enforced by various national and international bodies, with the primary aim of protecting public health and maintaining high-quality standards in the food industry.

A notable example is the Codex Alimentarius, created under the auspices of the Food and Agriculture Organization and the World Health Organization. The Codex Alimentarius provides a set of standards and guidelines aimed at food safety and quality, facilitating fair international trade and protecting consumer health.

Food safety regulations have been adopted at the insistence of those interested in addressing specific issues. For instance, the U.S. Congress adopted the "Pure Food and Drug Act" in 1906, which was later revised by the "Federal Food, Drug and Cosmetic Act." At the European level, in 1860, the British Parliament approved the "Food and Drink Adulteration Act."

ISO standards are consensus-based documents approved by recognized bodies that provide rules, guidelines, or characteristics for activities or their outcomes, aiming to achieve performance in a specific context. Standardizing specific requirements is necessary to establish the degree of product compliance.

The International Organization for Standardization (ISO) plays a significant role in developing and promoting international standards. ISO standards aid in uniformly conducting conformity verification activities across the industry and globally. Test reports and certificates resulting from the activities of accredited operators can be mutually recognized between countries without requiring additional tests, thereby enhancing international trade.

Consumers in industrialized countries have high demands for superior and consistent quality food products. To address these challenges, companies globally implement standardized quality assurance systems aimed at improving both the quality and safety of products and production processes.

Legislative requirements in the field of food safety and agro-food product quality testing lead to increased demand for products and services specific to these activities. Testing laboratories accredited according to ISO 17025 must demonstrate their competence by declaring measurement uncertainty and through interlaboratory tests.

In recent years, the European Union has been intensely active in developing tools for implementing food safety. Since 2000, the EU has issued regulations in this field, one of the first being Regulation (EC) No 178/2002.

In 2016, the International Organization for Standardization published ISO 17034:2016, replacing ISO Guide 35:2009. ISO 17034:2016 aligns with recent editions of conformity assessment standards, such as ISO 17025 (general laboratory quality) and ISO 17043 (proficiency testing).

ISO 17025 allows laboratories to demonstrate competence and capability to produce valid results, thereby strengthening confidence in their activities both nationally and internationally, facilitating the recognition of results across different states.

The increase in counterfeit food products and their impact on human health led to the establishment of the Knowledge Centre for Food Fraud and Quality by the European Commission in 2018. This center supports innovation in testing techniques through the development and standardization of new methods and improving measurement performance.[1]

Certified reference materials distributed by the Joint Research Centre (JRC) provide laboratories with the means to validate analytical methods and assess the accuracy of their measurement results. The JRC has developed a wide range of reference materials for food products, covering various analyte/matrix combinations.

In conclusion, both the improvement of quality of life and the smooth functioning of international trade depend on reliable measurements. In this context, there is intensive activity aimed at harmonizing tools for evaluating the quality of food products in EU member states, enhancing analytical performance and developing new food matrices used as certified reference materials.[2]

Reference materials have a wide range of applications in testing activities, including evaluating the quality of obtained results, calibrating analytical equipment and validating methods.

Chapter 2. The development of a quality system specific to producers of Certified Reference Materials

In the 1830s, railway transportation in the USA was plagued by accidents caused by train derailments due to wheel fractures. Cast iron manufacturers were accused of not supplying quality raw materials, while they claimed to deliver cast iron according to specifications. However, there was a need for a reference material for calibrating laboratory equipment. To

address the issue, the manufacturers collaborated with the Bureau of Standards, funding the development of the first reference materials in 1906. This initiative led to improved raw material quality and reduced accidents, demonstrating the importance of certified referenc materials in the industry.

According to ISO 17000:2004, conformity verification consists of demonstrating that the specified requirements for a system are met. [3]. These requirements are specified in normative acts such as regulations, standards and technical specifications. Accreditation is the process of recognition, through third-party auditing, of the fulfillment of managerial and technical quality requirements of the ISO standards requested by the entity being evaluated for conformity. Each European state has at least one nationally recognized body that performs conformity assessments. These bodies are also internationally recognized as members of the International Accreditation Forum (IAF).

According to ISO 17034:2016 "Reference material is a generic term applied to a material that is sufficiently homogeneous and stable with respect to one or more specified properties and has been established to be fit for its intended use in a measurement process". [4]

The characteristics can be quantitative or qualitative and the uses may include the calibration of measurement systems, the evaluation of measurement procedures, the assignment of values to other materials and quality control.

For the accreditation of the quality system, according to ISO 17034, the following documents must be developed:

- Detailed quality policy in the Quality Manual;
- Process scheme for the system, translated into process diagrams;
- Third-party audit reports on the fulfillment of authorization conditions;
- Necessary authorizations for operation according to applicable regulations;
- Certifications of personnel competence for the functions performed (conducted by an accredited service provider according to EN ISO/IEC 17024);
- General, operational and specific procedures, as well as work instructions associated with the procedures, all developed according to ISO 17034 (assimilated to product conformity assessment).

In conclusion, a quality management system accredited according to an international ISO standard must be traceable and procedurally defined.

Therefore, an important activity in the development of the QMS specific to CRM producers, as specified in the international standard ISO 17034, is the identification of the requirement, its classification to determine the group it belongs to (general procedures (GP), operational procedures (OP), specific procedures (SP)), coding and document development.

According to ISO 17034, the following activities are considered essential for reference material producers[5]:

- Formulation and adoption of policies;
- Development of testing, calibration and measurement methods, processes and procedures;
- Selection and qualification of technical personnel;
- Analysis of orders and contracts;
- Assignment of tasks within planned activities;

– Sampling, testing, calibration, measurement and examination;
– Analysis, approval and decision regarding results.

The diagram in Figure 2.1 illustrates the sequence of steps in the production process of reference material (RM) according to the ISO 17034 standard. The process begins with project planning, followed by the selection and acquisition of raw materials and their storage under controlled conditions (T=25°C, Umax=60%). This is followed by initial homogenization (D=100 mm, time=1 min), division and secondary homogenization (110 rpm, max time=5 min). Subsequent stages include bottling, induction sealing, labeling and final storage.

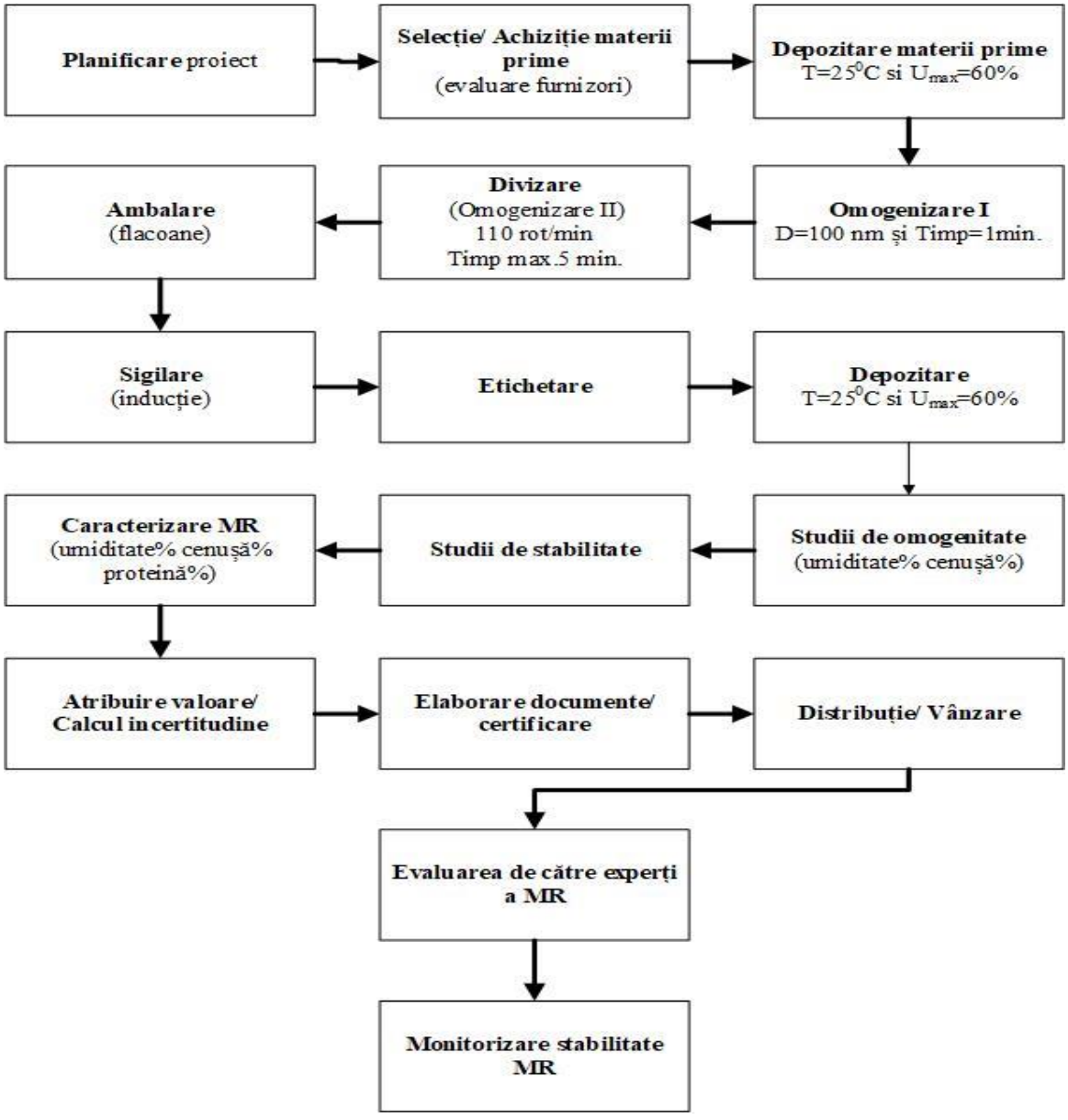

Fig. 2.1. Diagrama de procese pentru producerea MR, conform standardului ISO 17034

In addition to the human resources necessary for the development of reference material production activities, according to requirement 7.5.2 of the ISO 17034 standard, the CRM

producer must also ensure material resources, namely the technological spaces and equipment required for the realization of the products declared during the accreditation phase.

Technology has revolutionized quality management systems by optimizing the processes of planning, implementation and quality monitoring. The use of modern technological solutions allows organizations to collect and analyze data much more quickly and with increased accuracy, significantly reducing human errors and ensuring complete traceability of information.

Additionally, technology supports data-driven decision-making, which is essential for continuous improvement within Quality Management Systems (QMS). Efficient data collection and interpretation enable organizations to quickly identify deficiencies, optimize processes and implement effective continuous improvement practices.

One of the biggest challenges in laboratories that digitalization can address is the efficient management of data. The increase in digitalization is driven by several factors, such as the transfer of data resulting from studies conducted between laboratories, the increase in workload and the emphasis on data integrity from the initial stages of research.[6]

In general, organizations use Enterprise Resource Planning (ERP) software or other systems for processing customer orders, from receipt to completion. A quality management system can integrate most requirements with the help of ERP software.

In the field of reference materials, specialized software has been developed to support the production process. One such software is SoftCRM, produced by the National Institute of Organic and Pharmaceutical Chemistry, part of the Hellenic Research Foundation in Greece.

SoftCRM is a specialized software developed to facilitate the certification process of certified reference materials, comprising three main modules: homogeneity study, stability study and certification exercise.

SoftCRM offers advanced functionalities for handling and evaluating statistical data, reducing the risk of errors by facilitating the electronic transmission of data and automating their processing. The software includes rigorous statistical tests for detecting outliers, assessing data normality and analyzing variance, ensuring the validity and accuracy of results.

Chapter 3. Conclusions on the Current State of Quality Management in the Food Industry

The analysis of the current state of quality management in the food industry highlights the importance of optimizing it to ensure product compliance and improve operational efficiency. Strict regulations imposed by authorities, such as those concerning food hygiene, quality control and risk management, are essential for protecting public health. International and European regulations, established by organizations such as Codex Alimentarius, FAO and WHO, aim to maintain high standards of food safety and quality.

Combating food fraud is crucial for ensuring product integrity and protecting consumers. Food fraud, which involves adulterating, substituting, or manipulating food products for dishonest financial gain, can have severe public health consequences. Strict monitoring and control measures, the use of advanced testing technologies and increased transparency in the supply chain are essential for preventing and managing this issue.

Additionally, the growing trend of consumers seeking healthy and high-quality food products has compelled companies to adhere to strict standards to meet market demands. International initiatives such as SAFEFOODS, the Food Quality Initiative and METROFOOD significantly contribute to improving food quality and safety by developing innovative solutions and promoting best practices in the agro-food industry.

Chapter 4. Directions, Main Objective and Research-Development Methodology for New Reference Materials in the Agro-Food Sector

Chapter 4 identifies the research-development directions in the field of agro-food reference materials, focusing on improving food quality and safety, harmonizing analytical methods globally and supporting technological innovation. New reference materials are essential for calibrating equipment and verifying the accuracy of analytical results, facilitating the comparability and reproducibility of results between laboratories. They support the implementation of uniform quality control and food safety practices and contribute to evaluating new food technologies and adapting to changes in food composition.

The main objective of the research-development activity is the development and production of a multi-parameter cereal reference material, according to the SR EN ISO 17034:2017 standard, using wheat flour as the raw material. The research methodology involves documenting and integrating the requirements of the ISO 17034 standard, risk analysis, identifying the optimal raw material, creating experimental batches, evaluating the homogeneity and stability of the reference materials, testing optimal transport conditions and assigning informative values. Implementing a continuous quality monitoring system ensures the maintenance of the integrity and consistency of the reference materials throughout their use.

Chapter 5. Experiments on the production and quality evaluation of reference material

The ISO 17034 standard clearly specifies that homogeneity must be evaluated for each property of interest and that measurement uncertainty must be calculated for value certification.

The homogeneity study is necessary for all RM characterization schemes because it provides information about possible inhomogeneity variations, the presence of impurities, or deficiencies in the production of granular reference materials.

Homogeneity refers either to the variation of a property value between RM units or to the variation of the value within each unit. According to ISO Guide 35, the evaluation of between-unit variation is always necessary.

The number of candidate RM units evaluated must comply with the recommendations of ISO Guide 35 regarding the random selection of samples tested to determine the degree of homogeneity.

Thus, for a homogeneity study, depending on the lot size, between 10 and 30 units are randomly selected using the Research Randomizer application.

The homogeneity study was conducted for lot 1 and three units were analyzed, namely: P1, P5 and P8, randomly selected using the Research Randomizer application.

The results of the homogeneity studies for RM001F – IBA Wheat Flour are presented in Table 5.1 and are in accordance with requirement 7.10 of the international standard ISO 17034.

Tabel 5.1. Homogeneity study for the first batch of MR001F-IBA [7]

No.	Unit (Sample)	Dry mass (%)	Humidity (%)
1	P1(C4)	89.75	10.25
2	P1(C5)	89.86	10.14
3	P1(C6)	89.80	10.20
4	P1(C7)	89.85	10.15
5	P1(C9)	89.76	10.24
6	P5(C1)	89.67	10.33
7	P5(C2)	89.64	10.36
8	P5(C3)	89.67	10.33
9	P5(C4)	89.66	10.34
10	P5(C3')	89.74	10.26
11	P8(C2)	89.61	10.39
12	P8(C5)	89.62	10.38
13	P8(C7)	89.55	10.45
14	P8(C8)	89.71	10.29
15	P8(C9)	89.67	10.33

Table 5.1 presents the results of the homogeneity study for the first batch of reference material MR001F-IBA, evaluating the dry mass and moisture content for 15 randomly selected sample units. The dry mass values range between 89.55% and 89.86%, while the moisture content varies between 10.14% and 10.45%.

These results indicate good homogeneity within the batch, as the variations between samples are minimal. The low variability in moisture content, ranging from 10.14% to 10.45%, suggests that the reference material is consistent and homogeneous. Additionally, the constant dry mass values, ranging from 89.55% to 89.86%, confirm the uniformity of the material.

The interpretation of these data shows that the reference material MR001F-IBA has adequate homogeneity for use in analytical applications, ensuring reliable and replicable results. The study complies with the requirements of ISO 17034, demonstrating that all units of the reference material can be considered "identical" in terms of the measured properties.

Using the procedure for measuring moisture in wheat flour provided information on both between-unit and within-unit variability.

According to recommendation 6.7, Stability Assessment, from ISO Guide 35, reference materials must be sufficiently stable for their intended use, so that the end user can rely on the assigned value at any time during the validity period of the certificate.

Figure 5.1 presents the results of the stability study, under storage conditions, conducted for MR001F-IBA-Wheat Flou.

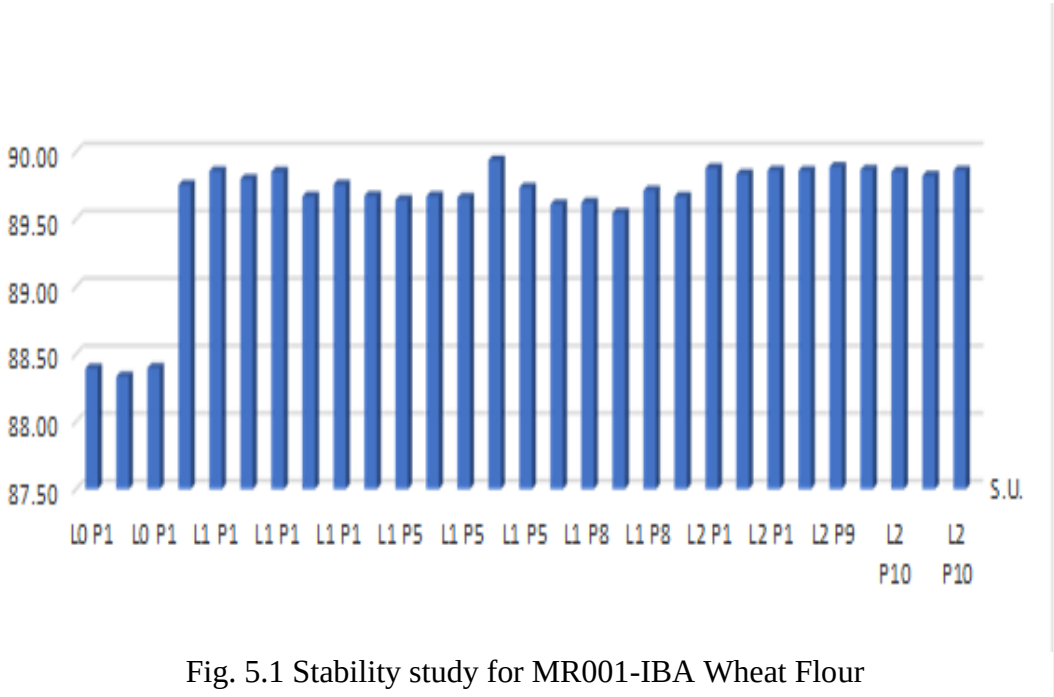

Fig. 5.1 Stability study for MR001-IBA Wheat Flour

Table 5.2 presents the variation in moisture content during storage for the first batch of MR001F-IBA Wheat Flour. The results are used to determine the shelf life of the reference material.

Tabel 5. Cariation in moisture content during storage for the first batch of MR001F-IBA Wheat Flour

Sample	Dry mass (%)	Humidity (%)	Difference from Mean (%)	Mean (%)
P1(C4)	89.75	10.25	0.05	10.2
P1(C5)	89.86	10.14	-0.06	10.2
P1(C6)	89.80	10.20	0	10.2
P1(C7)	89.85	10.15	-0.05	10.2
P1(C9)	89.76	10.24	0.04	10.2
P6(C12)	89.41	10.59	0.02	10.57
P6(C13)	89.45	10.55	-0.02	10.57
P3(C11)	89.62	10.38	-0.01	10.39
P3(C12)	89.60	10.40	0.01	10.39
P4(C12)	89.85	10.15	-0.04	10.19
P4(C11)	89.76	10.24	0.05	10.19

From Table 5.2 and Figure 5.2, it can be observed that the difference in moisture content varies between 0.00 and 0.04. This difference falls within the 0.1% limit for repeatability indicated in the vial (heterogeneity), according to the SR EN ISO 712:2010 standard.

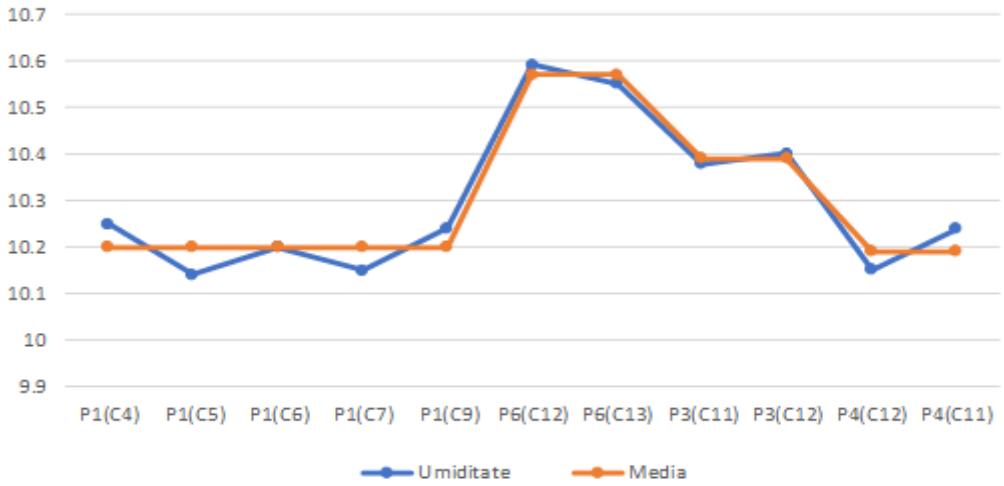

Fig. 5.2 Variația stabilității în depozitare a lotului 1 MR001F-IBA Wheat Flour

The moisture content for the 11 units ranges between 10.14% and 10.59%, with differences from the mean ranging between -0.06% and 0.05%. The mean moisture content for these samples is 10.2%, except for a few samples that show slightly higher values, such as P6(C12) and P6(C13), with moisture contents of 10.59% and 10.55%, respectively. These results indicate that the reference material maintains its stability under storage conditions, with minimal variations in moisture content. The differences from the mean are very small, suggesting that the reference material can be considered stable in the long term.

Chapter 6. Experiments on the Stability of the Reference Material MR001F-IBA

To produce a reference material, an experimental stability study is required according to the 17034:2017 standard. This study determines whether the value of the properties for which the candidate reference material has been characterized varies or degrades due to the environmental conditions to which it has been exposed, such as temperature, humidity and light.

According to ISO Guide 35:2017, it is necessary to understand three types of storage conditions for reference materials:

- Long-term storage conditions at the reference material producer's working unit;
- Short-term storage conditions required for the transport of the reference material;
- Storage and usage conditions of the reference material at the final user's working unit.

Testing a reference material for stability and estimating its shelf life requires conducting a long-term stability study under specified storage conditions for a minimum duration of 12 months.

To determine the stability of the property values of the reference material MR001F-IBA, a classic stability study was applied. This involves analyzing units of reference material from the same batch (produced simultaneously) under identical conditions at predetermined time intervals, according to the scheme outlined in ISO Guide 35:2017 (Figure 6.1).

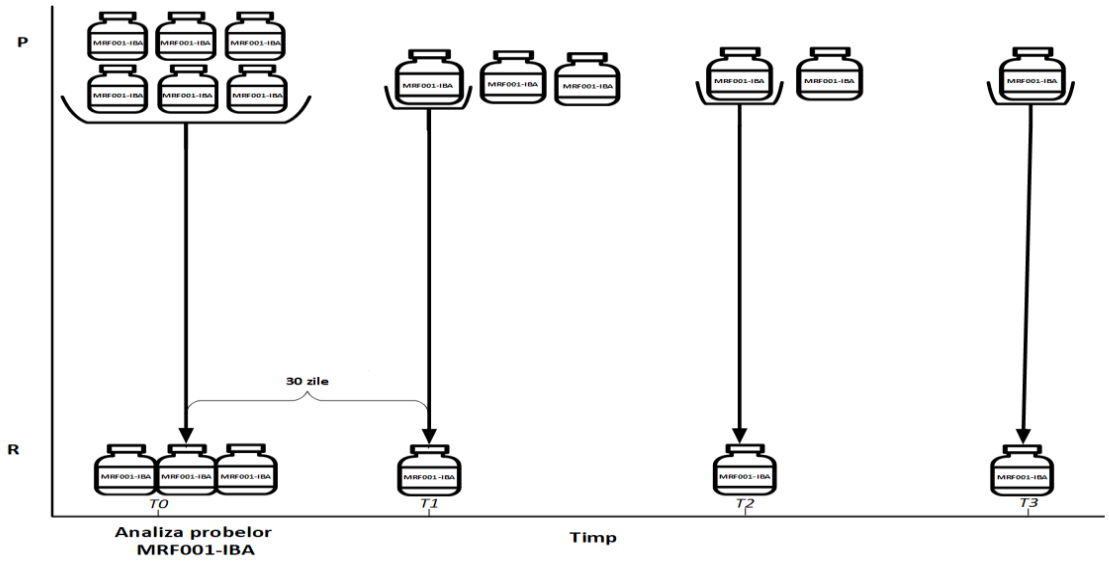

Fig. 6.1 Scheme of the classic stability study conducted on MRF001-IBA

For testing the two parameters of wheat flour, the candidate reference material produced by the National Institute of Research and Development for Food Bioresources - IBA Bucharest was used. The determinations were performed on two batches produced from the same raw material, under the same conditions. The same process was followed for producing both batches as was used for the first batch.

Evaluation of the results obtained for moisture content in the second batch

Using the previously mentioned method, six samples from the second batch of reference material were analyzed. At the time of producing the candidate reference material batch MR001F – IBA Wheat Flour, three samples were randomly chosen for initial analysis, also known as T0. These samples were included in the homogeneity study, while the remaining samples were analyzed at one-month intervals.

The results of the stability study are presented in Table 6.1.

Table 6.1 Results of the stability study for the moisture parameter in the second batch[8]

Crt.	*T0*			*T1*	*T2*	*T3*
Proba	L2.1	L2.9	L2.10	L2.2	L2.3	L2.4
1.	10.12	10.14	10.15	10.18	10.21	10.22
2.	10.17	10.11	10.18	10.24	10.26	10.19
3.	10.14	10.13	10.14	10.20	10.24	10.18
Medie	10.14	10.13	10.15	10.21	10.24	10.20
Deviația standard	0.025	0.015	0.021	0.031	0.025	0.021

To determine if there are significant differences in moisture content among the six analyzed CRM units, a one-way analysis of variance (ANOVA) was applied (Table 6.2).

Table 6.2 ANOVA analysis on moisture content on the second batch [8]

	DF[a]	Sum of Square[b]	Mean Square[c]	F [d]	P-value[e]
Grup (between groups)	5	0.026711	0.005342	9.713131	0.000671
Residual (within groups)	12	0.0066	0.00055	NaN	NaN
Total	17	0.333133	0.0019595		

The results indicate a statistically significant difference in the moisture means among the analyzed CRM samples. From Table 6.4, we can observe that the F statistic exceeds the critical F value and the p-value is 0.000671, which means the p-value is smaller than the significance level α of 0.05, indicating a 95% confidence level. Therefore, it is concluded that the null hypothesis is rejected, resulting in significant differences among the means of the six groups.

Evaluation of the results obtained for moisture content in the third batch

Table 6.3 presents the values obtained from the determinations performed on the third produced batch. As with batch two, three samples were randomly selected for T0 to be analyzed and used in the homogeneity study. The analysis of the subsequent samples, at T1 and T2 points, was also conducted at 30-day intervals.

Table 6.3 Humidity content in the third batch of candidate reference material

Crt.	*T0*			*T1*			*T2*	
Proba	**L3.2**	**L.3.12**	**L3.10**	**L.3.14**	**L.3.3**	**L3.5**	**L.3.6**	**L3.8**
1.	11.22	11.06	11.19	11.04	11.13	11.25	11.37	11.28
2.	10.91	10.96	11.02	10.96	11.41	11.10	11.38	11.27
3.	11.4	10.96	11.08	10.96	11.25	11.31	11.35	11.3
Medie	11.18	10.99	11.10	10.99	11.26	11.22	11.37	11.28
DV	0.248	0.058	0.086	0.046	0.140	0.108	0.015	0.015

For this batch, a one-way analysis of variance was also used to determine if there are significant differences among the group means. The results are presented in Table 6.4.

Table 6.4. ANOVA analysis on moisture content on the third batch of candidate reference material

	DF	Sum of Square	Mean Square	F Statistic	P-value
Grup (between groups)	7	0.398667	0.056952	4.288852	0.007557
Residual (within groups)	16	0.212467	0.013279	NaN	NaN
Total	23	0.611134	0.070231		

In Table 6.4, it can be observed that the F statistic exceeds the critical F value as in the previous case and the p-value is well below the 0.05 threshold, specifically 0.0075.

As with batch two, these results indicate that there are significant differences among the eight compared groups, highlighted by the graphical representation shown in Figure 6.2.

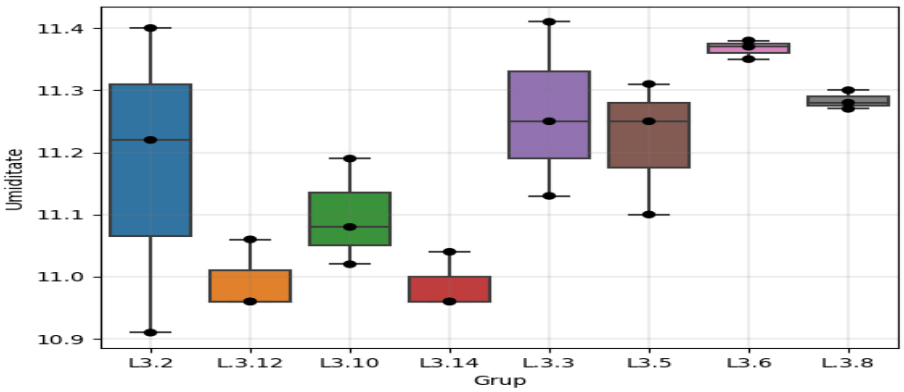

Fig. 6.2. ANOVA analysis on moisture content on the third batch

Stability study on ash content

For the analysis of the ash content in the wheat flour used in the production of CRM, the reference method for determining ash content by incineration was chosen, according to ISO 2171:2007.

The determinations for ash content using the reference method described above were performed on the same candidate reference material units as for moisture, with the goal of creating a multi-parameter reference material.

Evaluation of the results obtained for ash content in the second batch

The results obtained from the analysis of the reference material units regarding the ash content in the wheat flour are presented in Table 6.5.

Table 6.5.Results obtained for ash content in the second batch [9]

Crt.	*T0*			*T1*	*T2*	*T3*	*T4*
Proba	L2.1	L2.9	L2.10	L2.15	L2.2	L2.3	L2.4
1.	0.54	0.52	0.52	0.54	0.52	0.50	0.56
2.	0.50	0.50	0.55	0.56	0.53	0.53	0.53
3.	0.55	0.52	0.54	0.53	0.50	0.55	0.53
Medie	0.53	0.51	0.54	0.54	0.52	0.53	0.54
Deviația standard	0.026	0.012	0.015	0.014	0.015	0.025	0.017

Table 6.6 presents the results of the analysis of variance for ash content, from which it can be observed that there are no significant differences between the group means analyzed.

Tabel 6.6 ANOVA analysis on ash content on the second batch of candidate reference material

	DF	Sum of Square	Mean Square		F Statistic	P-value
Grup (between groups)	6	0.002362	0.000394		1.117	0.401041
Residual (within groups)	14	0.004933	0.000352		NaN	NaN
Total	20	0.007295	0.000746			

Due to the fact that the p-value is 0.4010, which is greater than the confidence level (α = 0.05) and the F-statistic does not exceed the critical value, we cannot reject the null hypothesis that the means of the seven analyzed groups are equal.

Evaluation of the results obtained for ash content in the third batch

Since batch three is produced from the same raw material as batches 1 and 2, which have demonstrated both homogeneity and stability in terms of the ash content of the wheat flour from which the candidate reference material MR001F-IBA is produced, it was decided to increase the interval for determining the ash content for this batch.

To determine the optimal storage conditions for the candidate reference material, batch 3 was randomly divided into two groups stored under different environmental conditions: the first group was stored at a temperature of 4°C and the second group at a temperature ranging between 25°C and 30°C.

Table 6.7 presents the results obtained at T0 and T1 for the ash content in batch 3.

Table 6.7 Results obtained for ash content in the third batch [9]

Crt.	T0		T1	
Proba	L3.10C	L3.2F	L3.5 C	L3.3F
1	0.51	0.51	0.56	0.51
2	0.48	0.49	0.55	0.54
3	0.52	0.53	0.54	0.52
Media	0.51	0.51	0.55	0.52
Deviația Standard	0.0110	0.0204	0.01221	0.0168
*C – interval temperatură 25 – 30 °C *F – temperatură stabilă 4°C				

For data interpretation, analysis of variance (ANOVA) was again chosen to check if there are significant differences among the four samples (Table 6.8).

Table 6.8 ANOVA analysis on ash content on the third batch of candidate reference material[9]

	DF	Sum of Square	Mean Square	F Statistic	P-value
Grup (between groups)	3	0.003292	0.001097	3.376068	0.074961
Residual (within groups)	8	0.0026	0.000325	NaN	NaN
Total	11	0.005892	0.001422		

From the data resulting from the analysis of variance, it can be observed that the p-value is greater than α and the F-statistic does not exceed the critical F value of 4.066. This means that the null hypothesis cannot be rejected, indicating that there are no significant differences between the means of the four analyzed groups.

Figure 6.3 graphically presents the results of the analysis of variance for batch 3 regarding ash content.

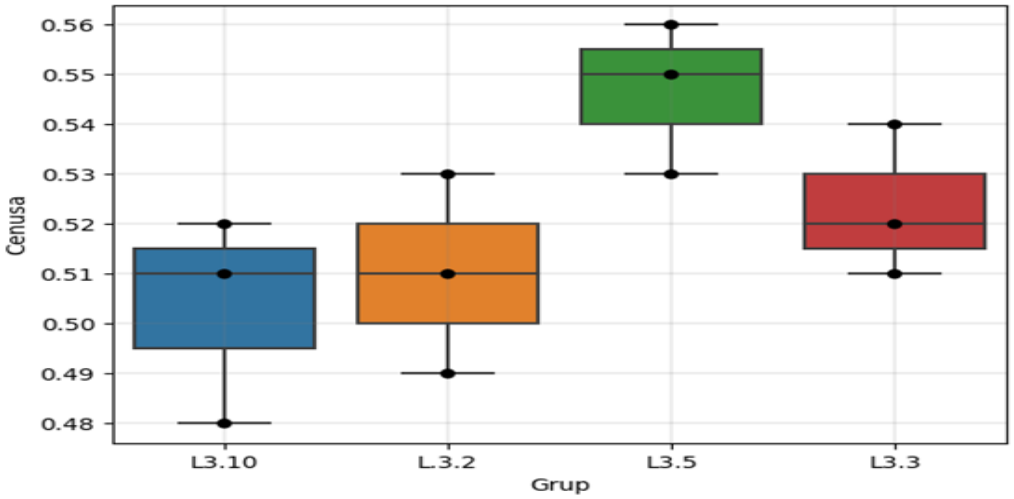

Fig. 6.3 Analysis of variance for batch 3 regarding ash content.

Chapter 7. Analysis of risk factors impacting the development and production of reference materials

In the first part, chapter seven explores risk management in the development of reference materials, highlighting the importance of effective management to ensure safety, compliance and operational efficiency in laboratories. Identifying hazards, assessing risks and implementing mitigation measures are essential for preventing accidents and ensuring the integrity of experiments. Methods such as Failure Modes and Effects Analysis (FMEA) and the 5X5 L Matrix Method are frequently used for risk management in laboratories. The implementation of computerized risk registers has improved the monitoring and management of risks, ensuring continuous improvement of safety protocols, especially in unprecedented situations such as the COVID-19 pandemic.

Risk analysis is crucial in the use and management of reference materials to ensure their quality. Identifying threats and vulnerabilities, such as contamination and degradation of reference materials, is essential for maintaining the integrity and accuracy of data. Risk assessment involves analyzing the effects and probabilities of risks, using techniques such as risk assessment matrices, Hierarchical Task Analysis (HTA) and HAZOP analysis. The ISO 31000:2018 standard provides a global framework for risk management, promoting a systematic and comprehensive approach. In the context of reference materials, risk management involves identifying, evaluating, controlling and monitoring the risks associated

with development, production and distribution, thereby ensuring their quality, accuracy and stability.

The process of identifying and analyzing risk factors for the reference material MR001F – IBA was guided by structured methodologies such as HTA and SWOT analysis. Each stage of the production process was analyzed to identify associated risks, highlighting vulnerable points and major risks such as measurement errors and fluctuations in raw material quality. Identifying and anticipating these risks are essential for ensuring the long-term quality and stability of the product.

By using the HTA method, it was possible to gain a comprehensive understanding of the risks associated with the development of the reference material and to formulate appropriate strategies for managing and mitigating these risks.

In the development and production of reference materials, it is crucial to properly identify and classify risks, considering their potential impact on the objectives and performance of the development, production and usage process.

Thus, a structured approach compliant with the ISO 31000:2018 standard is essential for effective risk management in this critical field. By classifying risks into categories such as technical, operational and compliance risks, laboratories can focus their efforts and resources on key aspects and develop specific management strategies to minimize the consequences on the development and production process of reference materials.

The classification of risks in the development and production of the reference material MR001F – IBA can be carried out based on their nature and potential impact on the process. Thus, the risks identified using the HTA method can be divided into the following main categories presented in Table 7.1:

Table 7.1 Risks associated with the development of the reference material MR001F-IBA

Type of risk	**Associated risks**	**Impact/Probabilty/ Priority**	**Preventive or mitigating measures**
Technical Risk	Variability in raw material quality	Impact: Medium Probability: High Priority: Medium	Implement a raw material quality monitoring system to ensure compliance with required specifications.
	Variability in process parameters	Impact: Medium Probability: Medium Priority: Medium	Closely monitor and control process parameters. Implement calibration and adjustment procedures for equipment to maintain parameters within limits.
	Technological processing issues	Impact: High Probability: Medium Priority: High	Regularly supervise and maintain equipment to prevent failures and maintain technological process efficiency.
	Measurement and control errors	Impact: Medium Probability: High Priority: Medium	Regularly verify and calibrate measurement and control equipment to ensure accuracy and reliability of results.

Type of risk	Associated risks	Impact/Probabilty/ Priority	Preventive or mitigating measures
	Process instability	Impact: High Probability: Medium Priority: High	Identify and eliminate sources of fluctuations in production processes to ensure product stability and consistency.
	Equipment and machinery defects	Impact: Medium Probability: High Priority: Medium	Implement a preventive maintenance program to prevent failures and ensure proper functioning of equipment and machinery.
	Degradation of reference material	Impact: Medium Probability: High Priority: High	Store and handle the material under controlled temperature and humidity conditions. Use appropriate packaging to prevent deterioration.
Operational Risks	Contamination with microbiological or chemical agents	Impact: Medium Probability: Medium Priority: Medium	Implement strict hygiene protocols in handling and production processes. Use properly sterilized equipment and surfaces.
	Incorrect handling or misuse of material	Impact: Mediu Probabilitate: Medie Prioritate: Medie	Provide adequate training to personnel on proper handling and use of the material. Implement clear and verifiable work procedures
	Equipment failures	Impact: Medium Probability: High Priority: Medium	Implement a preventive maintenance program to detect and resolve potential equipment failures.
	Logistics and supply chain management	Impact: Medium Probability: Medium Priority: Medium	Carefully plan and monitor the supply chain to avoid delays and deficiencies in raw material and equipment deliveries.
	Insufficiently trained personnel or non-compliance with operational procedures	Impact: High Probability: Low Priority: Medium	Provide adequate training and development to employees to ensure they are qualified and prepared to comply with operational procedures.
Compliance Risks	Inadequate use of reference material	Impact: High Probability: High Priority: High	Provide detailed instructions and usage guides for the material. Strictly supervise the use and application of the material according to established procedures.
	Non-compliance with quality regulations	Impact: High Probability: Medium Priority: High	Constantly monitor and update the legal framework and quality standards to ensure compliance with legislative requirements and regulations.

Type of risk	Associated risks	Impact/Probabilty/ Priority	Preventive or mitigating measures
	Non-compliance with safety and security standards	Impact: High Probability: Low Priority: Medium	Implement rigorous quality and safety control procedures to prevent incidents and ensure compliance with safety and security requirements.
	Labeling and packaging issues	Impact: Medium Probability: Medium Priority: Medium	Regularly review and update labeling and packaging procedures to ensure compliance with legal requirements and applicable regulations.
	Non-compliance with legal and contractual requirements	Impact: High Probability: Low Priority: Medium	Implement an efficient document management system to ensure record-keeping and compliance with legal and contractual requirements.

To create a complex and relevant tool for the food industry and research, a new property, gluten, has been assigned to the reference material MR001F-IBA. Gluten is an essential component of wheat flour and plays a crucial role in the baking process and the quality of bakery products. In the food industry, evaluating gluten content is important to ensure the quality of the final products.

Thus, the introduction of gluten as a parameter in the reference material initially based only on moisture and ash can be anchored in the needs of the food industry, the standardization of analyses, the complexity of wheat flour composition and adaptability to market demands.

In the context of developing a wheat flour reference material, conducting a feasibility study to determine the optimal gluten content is necessary to evaluate the stability of the raw material by selecting flour with consistent properties, which is essential for obtaining a reproducible and reliable reference material.

Raw material variability can significantly affect experimental results and the quality of the final product. The feasibility study allows for the identification of flour with uniform properties, essential for obtaining a stable and robust reference material.

To select the type of flour used as the raw material, an experimental feasibility study was conducted, evaluating five types of flour, packaged in 5 kg bags and supplied by the same producer.

Among the types of flour analyzed in the study, type 650 white wheat flour stands out due to its low variation level, characterized by low standard deviation values for gluten content, between 0.5 - 1.3%, compared to the other types of flour examined.

The results of the feasibility study conducted at the Food Chemistry Laboratory of INCD IBA-Bucharest are presented in Table 7.2.

Table 7.2 Wet gluten content for the types of flour analyzed [10]

Type of flour	Sample no.	R1, %	R2, %	R3, %	Medie*, %	SD	RSD (r), %
White flour type 480	1	37.5	37.0	36.2	36.9^{A}	0.7	1.8
	2	35.6	37.0	37.4	36.7^{A}	1.0	2.6
	3	37.3	35.8	35.7	36.2^{A}	0.9	2.6

White flour type 550	1	28.6	28.4	28.6	28.5^{A}	0.2	0.6
	2	28.3	28.5	28.7	28.5^{A}	0.2	0.6
	3	29.6	28.3	27.9	28.6^{A}	0.9	3.2
White flour type 650	1	27.7	27.8	28.0	27.8^{A}	0.1	0.5
	2	27.7	28.1	27.7	27.8^{A}	0.2	0.8
	3	28.3	28.0	27.6	28.0^{A}	0.4	1.3
Black flour type 1350	1	29.3	28.7	28.7	28.9^{A}	0.4	1.3
	2	29.0	29.4	29.7	29.4^{AB}	0.4	1.3
	3	30.3	29.9	29.7	30.0^{B}	0.3	1.1
Wholemeal flour	1	32.3	33.1	33.0	32.8^{A}	0.4	1.3
	2	33.1	32.3	32.6	32.7^{A}	0.4	1.2
	3	32.0	34.5	33.8	33.4^{A}	1.3	3.9

The production of the candidate reference material followed the same technological sequence as the previous batches. Thus, according to the technological flow described in diagram number 7.1, the acquired raw material was homogenized, sieved, packaged, sealed and labeled.

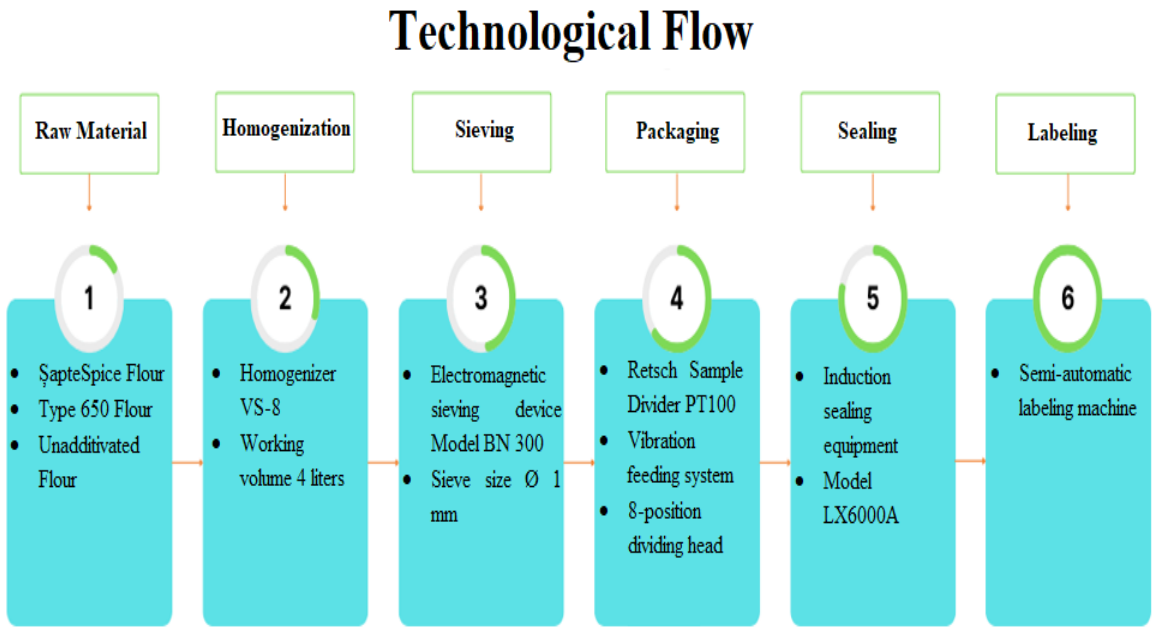

Fig. 7.1 Technological flow for the production of MR001F - IBA

Since previous experimental studies indicated a variation in moisture content in the batches of produced reference material, additional investigations were necessary to determine if the new property assigned to the wheat flour reference material is influenced.

Therefore, in this experimental study, a new batch of reference material obtained from type 650 wheat flour was analyzed. This batch was randomly divided into three equal fractions, each fraction being stored at one of the three proposed temperatures, namely ambient temperature (approximately 25°C), 4°C and -18°C.

The study was conducted over a period of 5 months, with three samples analyzed at 30-day intervals, each sample stored in one of the three established storage conditions with specific parameters.

To determine the homogeneity and stability of the attribute assigned to the candidate reference material, rheological analyses and scanning electron microscopy were performed to observe if there are differences in the structure of the reference material subjected to different storage conditions.

To determine the wet gluten content, the Glutomatic 2200 equipment shown in Figure 7.2 was used, applying the analysis described in the standard "ISO 21415-2:2015 Wheat and wheat flour — Gluten content — Part 2: Determination of wet gluten and gluten index by mechanical means." This analysis is performed by separating the protein substances from a dough prepared from the selected samples, using a sodium chloride solution for washing.

Fig. 7.2 Glutomatic 2200 cu Gluten Index Centrifuge 2015

The use of SEM for the analysis of wheat flour can provide several benefits and valuable information for research. This analysis allows for detailed visualization of the morphology of wheat flour particles, revealing information about the size, shape, structure and surface characteristics of these particles.

Scanning Electron Microscopy (SEM) is an advanced microscopy technique that uses an electron beam to obtain high-resolution images of the surfaces of objects.

Through this technique, researchers can gain a deeper understanding of the composition and distribution of particles in the flour matrix, thereby contributing to the evaluation and improvement of the quality of reference materials and their production processes.

Gluten is an important component of wheat flour, providing elasticity and the ability to rise during baking. The use of SEM can allow for the observation of the structure and distribution of gluten within the flour, providing information about the potential quality of the wheat flour.

This technique allows for the examination of particle structure and is useful in identifying impurities or contaminants that may affect the quality of the flour.

The examination of the reference material units was conducted using a scanning electron microscope, model Nova NanoSEM 630, manufactured by FEI Company, USA and available at IMT Bucharest (Fig. 7.3).

Fig. 7.3 Nova NanoSEM 630 Equipment

In the images resulting from the SEM analysis, fractions of the wheat grain endosperm can be observed, displaying starch matrices. These are three-dimensional structures composed mainly of starch granules embedded in a protein network. This protein network is formed from gliadins and glutenins, representing approximately 30% and 50% of the total wheat protein, respectively and is the one that surrounds and suspends the starch granules within the endosperm. The protein network is what provides elasticity and strength to the dough in the baking process.

Gliadins and glutenins tend to form fibrous and granular structures, aspects highlighted by the SEM analysis, while starch exhibits a predominantly spherical or ovoid structure in the form of granules. Areas that display a fibrous or granular texture might be indicative of the presence of gluten proteins.

Excluding gliadin and glutenin, the endosperm contains two other types of protein, albumin and globulin, which represent a smaller percentage compared to the other two. These types of protein do not have a distinct shape or visible structure in the flour, unlike starch granules or gluten proteins (gliadins and glutenins). Generally, these proteins are present as discrete molecules dispersed in the starch and gluten matrix, contributing to the overall protein composition of the flour.

When wheat grains are milled, the endosperm is separated from the outer layers and fragmented, generating a variety of flour particles.

Overall, the compositions of these particles predominantly consist of fractions of the endosperm (aggregates composed of complete endosperm cells), intact starch granules, damaged starch granules and uneven protein fragments.

Figure 7.4 presents pure starch (a), flour from the candidate reference material at the time of production (b) and vital gluten (c). The morphological analysis of pure starch and vital gluten using scanning electron microscopy serves as a tool for identifying these components in type 650 wheat flour.

In image (a) of Figure 7.4, the pure starch granules can be observed, which are easier to identify due to their spherical and sub-spherical shape with a smooth appearance that may exhibit slight indentations or grooves.

Image (b) of Figure 7.4 illustrates the candidate reference material at the time of production. In this image, the flour is characterized by a high concentration of large starch granules. The protein network is also visible, presenting a three-dimensional structure formed by gliadins and glutenins.

The third image (c) of Figure 7.4 shows vital gluten, highlighting its solid and cohesive structure, characteristic of gluten proteins.

According to the experimental study, three samples were analyzed at 30-day intervals, with each sample stored in different environmental conditions. The images resulting from these analyses, presented in Figures 7.5 to 7.8, illustrate the microstructural evolution of the candidate reference material throughout the study.

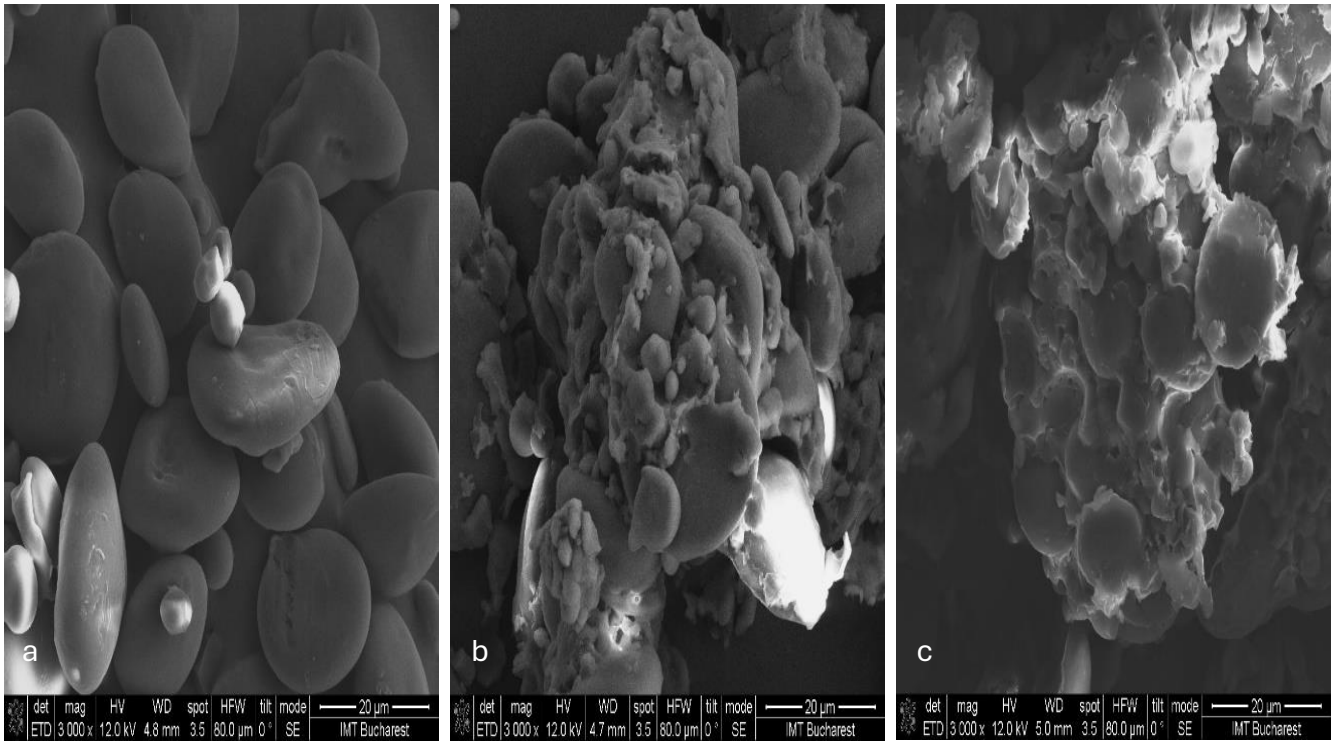

Fig. 7.4 SEM image analysis
a) pure starch b) wheat flour c) vital gluten

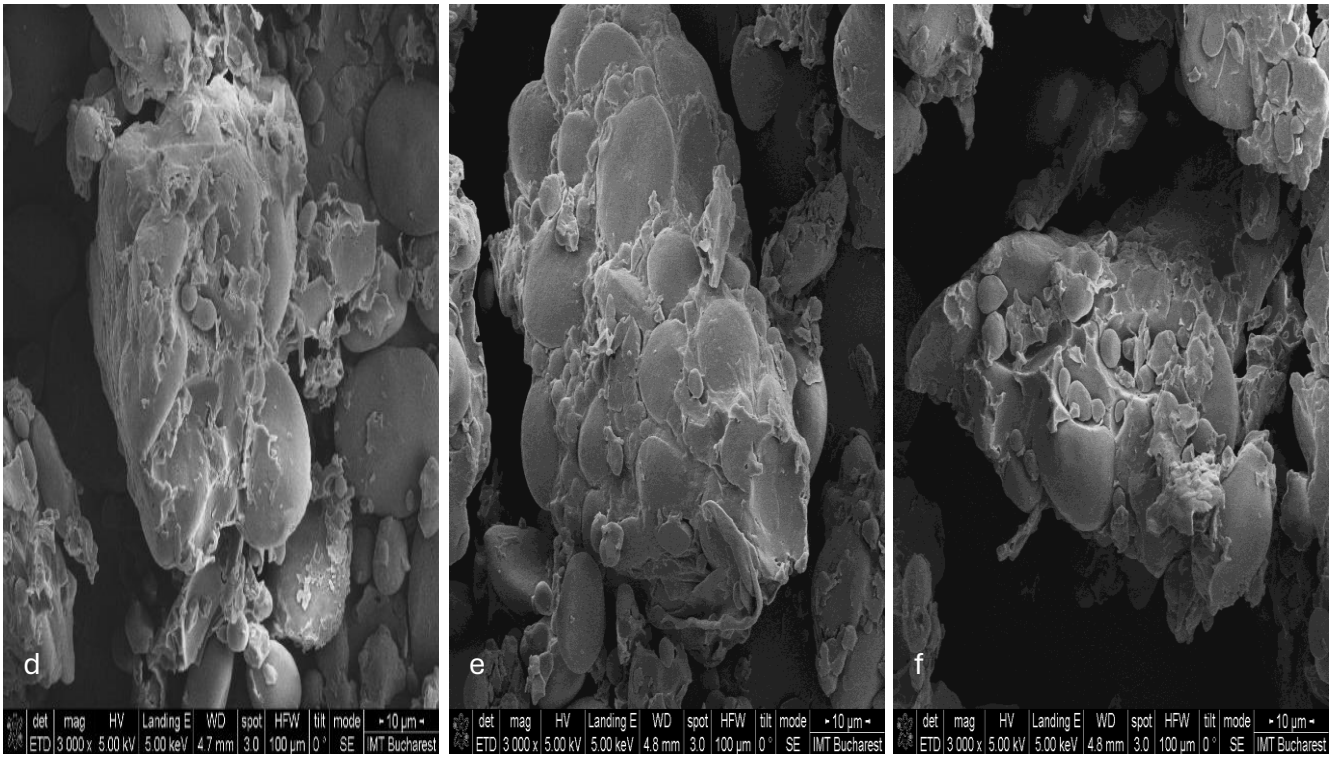

Fig. 7.5 SEM image analysis T1

d) wheat flour RT e) wheat flour 4°C f) wheat flour 18°C

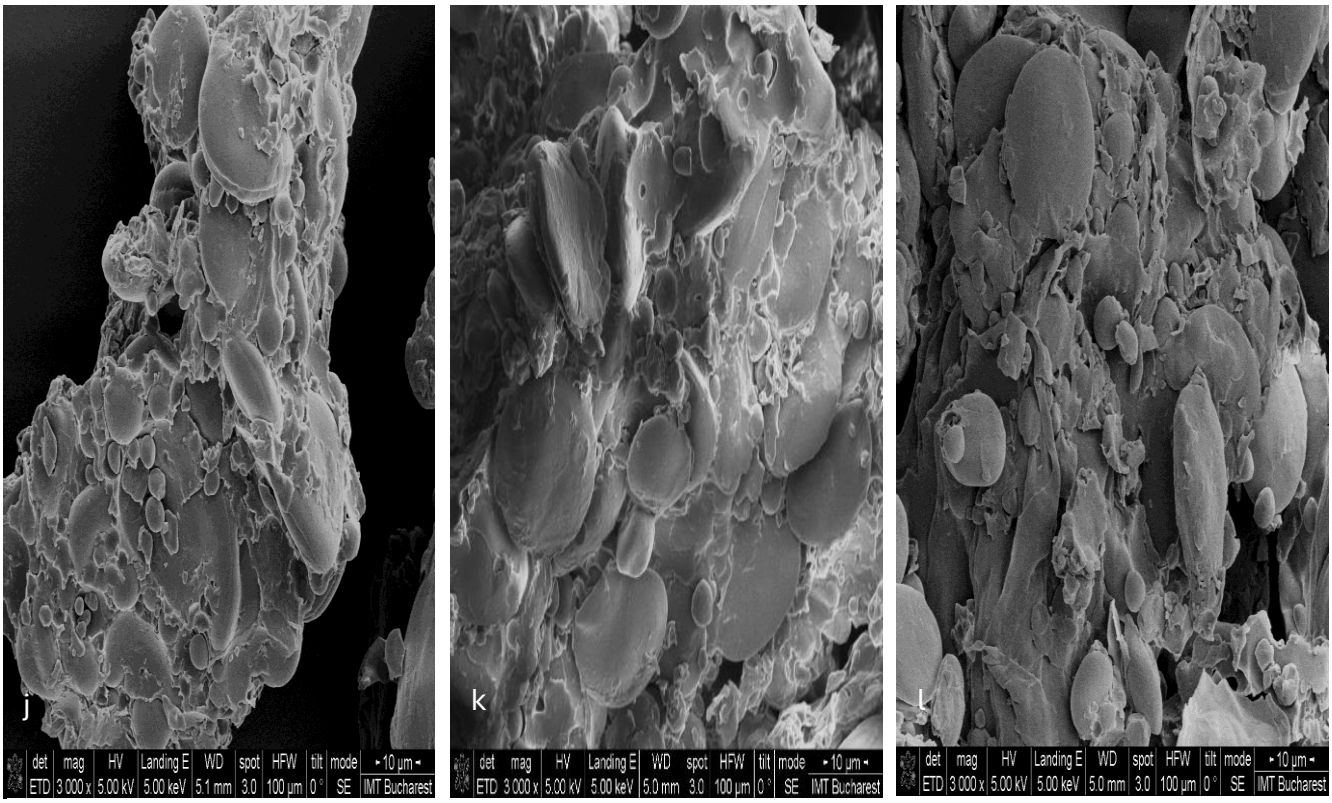

Fig. 7.6 SEM image analysis T2

a) wheat flour RT b) wheat flour 4°C c) wheat flour 18°C

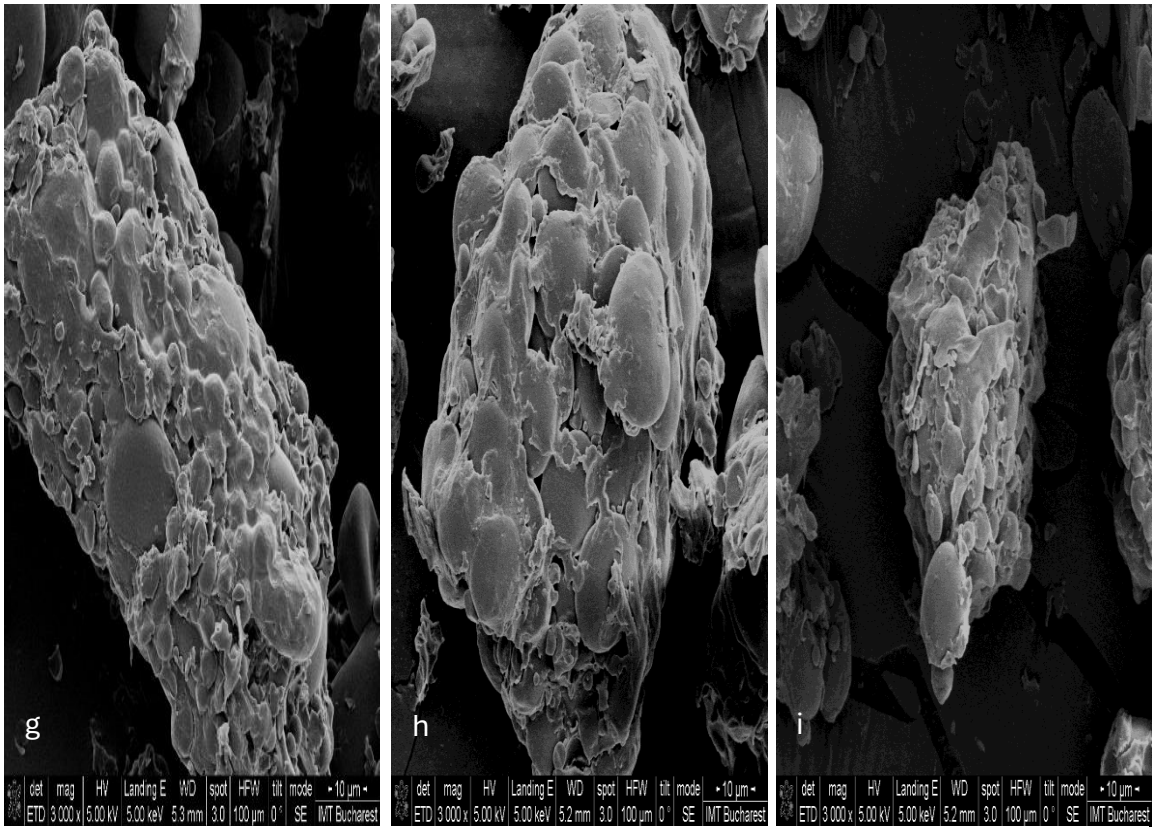

Fig. 7.27 SEM image analysis T3
a) wheat flour RT b) wheat flour 4℃ c) wheat flour 18℃

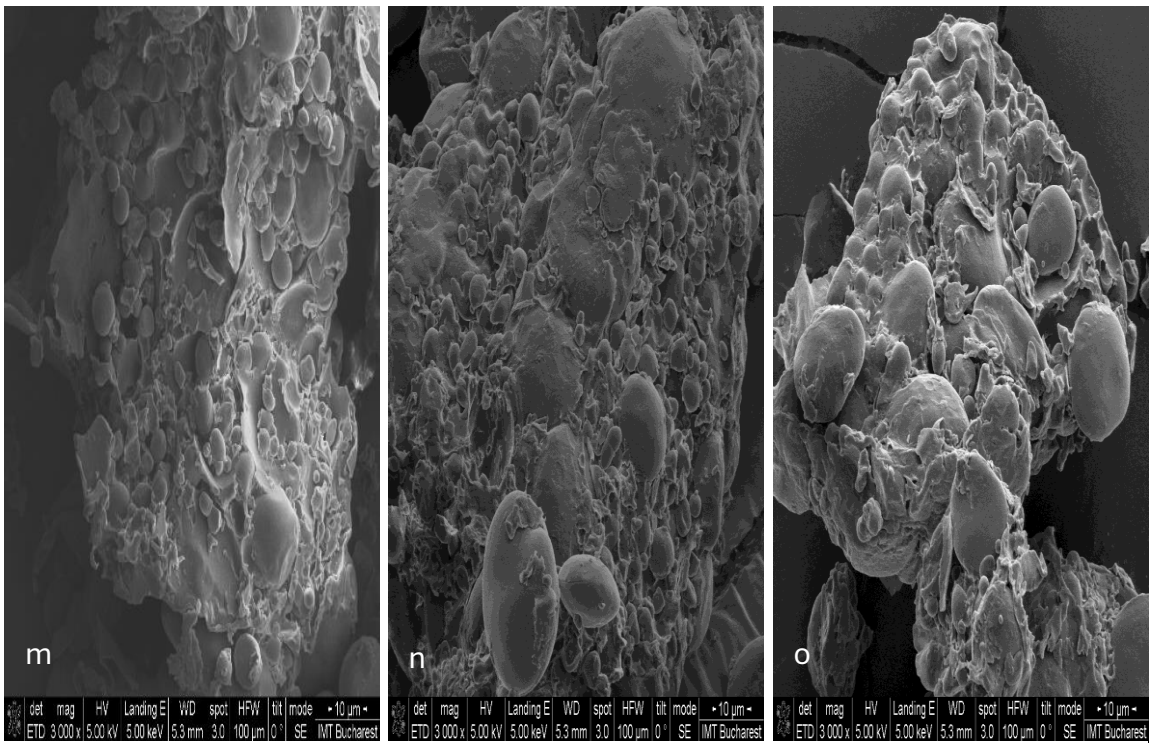

Fig. 7.8 SEM image analysis T4
a) wheat flour RT b) wheat flour 4℃ c) wheat flour 18℃

The SEM image analysis of the reference material produced from type 650 wheat flour stored under three different environmental conditions - ambient temperature (approximately 25°C), 4°C and -18°C - over a period of 5 months did not reveal major changes in the studied samples. This result suggests that type 650 wheat flour can be stored under these conditions for at least 5 months without its morphology being significantly affected at the microscopic level, according to the SEM analysis. This is important information suggesting that the structural stability of the flour can be maintained over a relatively long term under these storage conditions.

In addition to the SEM analysis of the reference material, rheological analyses were also performed to evaluate the behavior of type 650 wheat flour during storage under the three established conditions.

The results of these analyses, presented in Table 7.3, confirmed the observations made during the SEM analysis, showing that the rheological properties of the flour remain stable over the 5 months of storage at the aforementioned temperatures. These results indicate that type 650 wheat flour can maintain the same rheological characteristics in various environmental conditions and can be reliably used after longer periods of storage at variable temperatures.

Table 7.3 Results of the rheological analyses for type 650 wheat flour**

No.	Period	Temp.	Sample	Humidity	Wet Gluten	Gluten deformation index	Gluten index	Falling number
0	Initial	RT	0	12.83	31	3.5	92	389
1	2 luni	RT	1	11.14	31.4	2.5	92	442
2	2 luni	4C	2	11.16	31.6	4.5	88	392
3	2 luni	18C	3	11.12	32.3	5.5	86	376
4	3 luni	RT	1	9.52	31.9	4	83	416
5	3 luni	4C	2	9.52	32	3.5	84	433
6	3 luni	18C	3	9.5	30.6	3.5	82	415
7	4 luni	RT	1	12.6	31.5	2.5	90	436
8	4 luni	4C	2	12.83	31.5	5	85	381
9	4 luni	18C	3	12.51	30.3	4.5	84	426
10	5 luni	RT	1	13.13	31	3.5	85	441
11	5 luni	4C	2	12.88	31.2	3.5	85	403
12	5 luni	18C	3	13.09	31	4	84	386

The rheological analysis of type 650 wheat flour, conducted over a period of five months, highlights significant aspects regarding its behavior under various experimental conditions. The research was performed at three distinct temperatures, providing relevant data for evaluating its rheological properties.

The results of the rheological analyses regarding the wet gluten content were evaluated using statistical methods according to ISO 35:2017 guidelines. The main goal of this evaluation was to determine the stability of the new property assigned to the candidate reference material.

In analyzing the three data sets, outliers were identified using the interquartile range (IQR) method based on the calculated limits. The wet gluten content value determined at T0

for the packaged raw material was classified as an outlier and eliminated because it was below the lower limit of the range, both for the group stored at 4℃ and the group stored at -18℃.

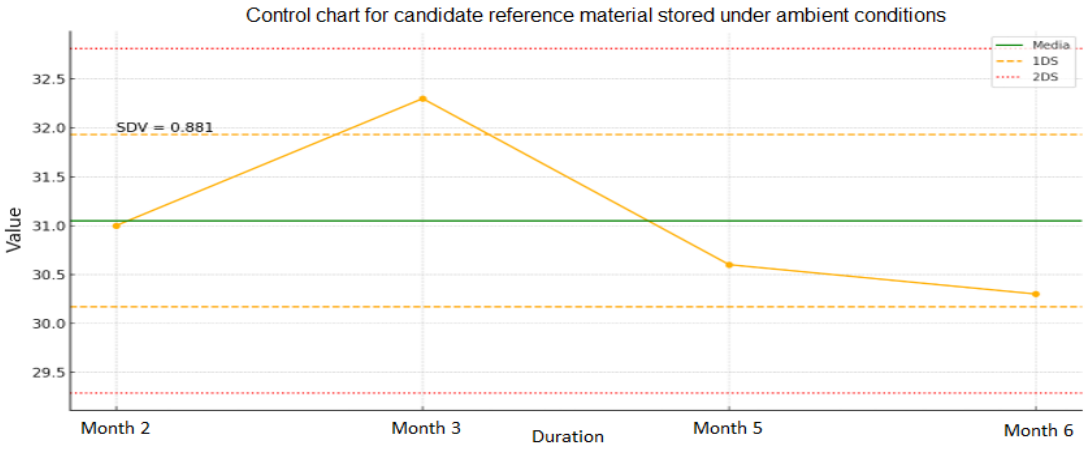

Fig. 7.9 Control chart for candidate reference material stored under ambient conditions

Figure 7.9 illustrates the control chart for RM stored under ambient conditions. The mean of the measured values is 31.55 and the standard deviation (SD) is 0.881, indicating moderate variability of the material. Most measured values are within the ±1SD limit, with one value close to ±2SD. This behavior suggests relative stability of the material under these conditions; however, there are trends of considerable variation, which may affect long-term consistency.

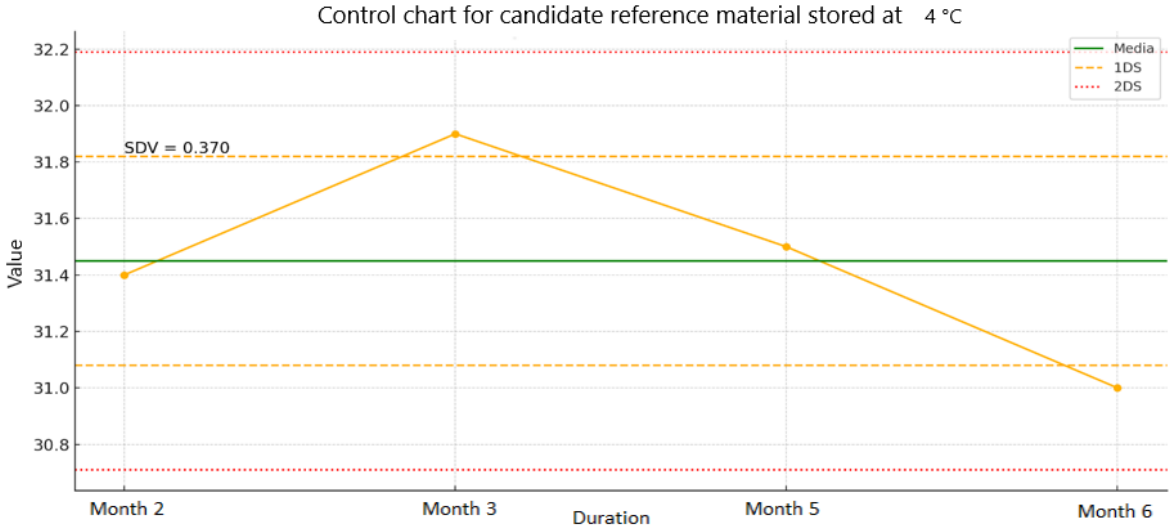

Fig. 7.10 Control chart for candidate reference material stored at 4 °C

Figure 7.10 presents the control chart for RM stored at 4°C. The mean of the measured values is 31.45 and the standard deviation (SD) is 0.370, indicating low variability. All values are within the ±1SD limit, signaling significant stability of the material at this temperature. This storage condition ensures high consistency and minimal variations, making it a favorable option for maintaining the material's properties.

Figure 7.11 shows the control chart for RM stored at -18°C. The mean of the measured values is 31.55 and the standard deviation (SD) is 0.330, indicating the lowest variability among all tested conditions. All measured values are within the ±1SD limits, reflecting optimal stability and minimal variability of the material at this temperature.

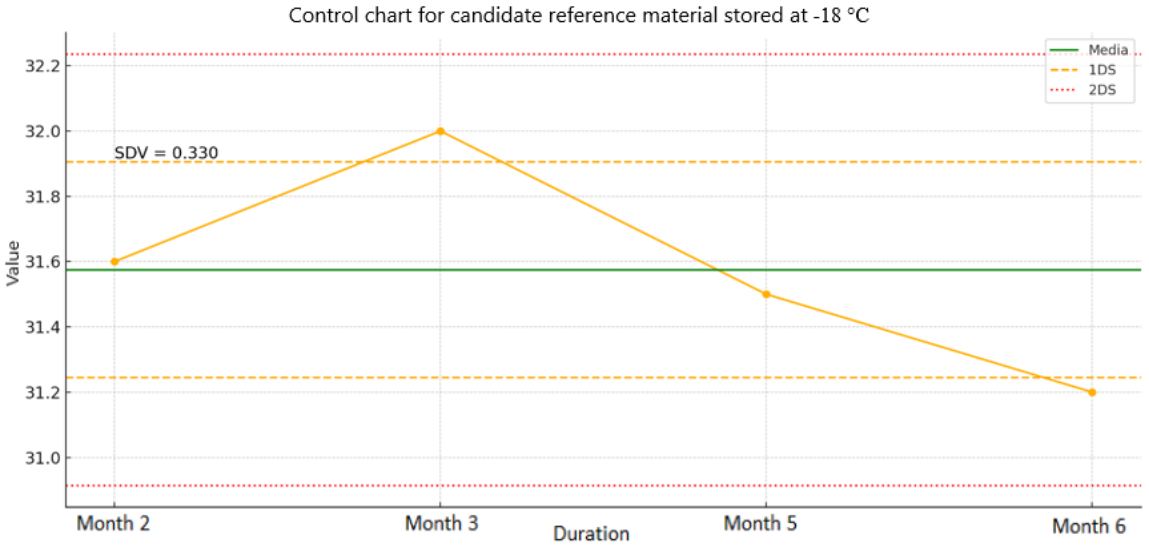

Fig. 7.11 Control chart for candidate reference material stored at -18 °C

These results suggest that storage at -18°C is the most favorable condition for preserving the material's properties, ensuring long-term stability.

Comparing the three storage conditions, it is found that low temperatures (4°C and -18°C) ensure reduced variability and superior stability of the candidate material (RM). Storage at -18°C is the most favorable, providing optimal conditions for maintaining the material's properties over the long term. Although relatively stable, ambient storage conditions present greater variability and potential for fluctuation, making it the least favorable among the three tested conditions.

ANOVA analysis was conducted to determine if there are statistically significant differences between the means of the wet gluten values from the three groups, providing a clear understanding of potential differences. The results of the ANOVA analysis are presented in Table 7.3.

Table 7.3. Results of the ANOVA analysis for the three groups

	Sum of Squares (SS)	**DF**	**Mean Square**	**F - value**	**P - value**
Between groups	0.882641	2	0.44132	0.882641	0.44666
Within groups	0.882641	9	0.098071		
Total	376.3	11	34.209091		

The p-value of 0.446660 is much higher than the significance threshold of 0.05, which means that there are no statistically significant differences between the analyzed groups (Fig. 7.12).

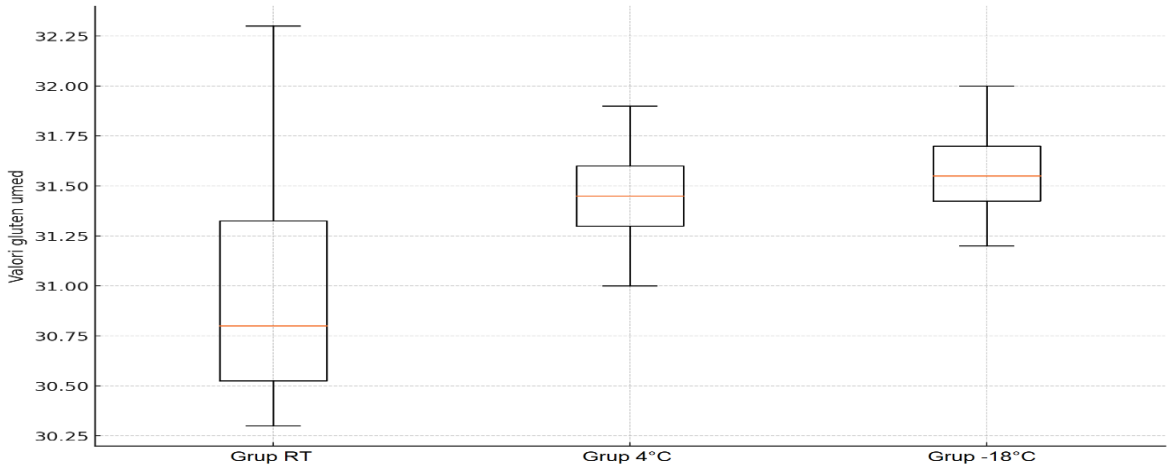

Fig. 7.12 Graphical representation of the ANOVA analysis results for the three groups

After ANOVA the Tukey test was applied to directly compare each pair of groups. The Tukey test helps to precisely identify which groups differ significantly from each other, providing additional details about the specificity of the observed differences. The results are presented in Table 7.4.

Tabel 7.4. Tukey HDS for the three groups

Grup A	**Grup B**	**meandiff**	**p-adj**	**lower**	**upper**	**reject**
Grup RT	Grup 4 °C	0.4	0.6133	-0.7526	1.5526	FALSE
Grup RT	Grup -18 °C	0.525	0.4444	-0.6276	1.6776	FALSE
Grup 4 °C	Grup -18 °C	0.125	0.951	-1.0276	1.2776	FALSE

All p-adj values are considerably higher than the significance threshold of 0.05 and the "reject" column specifies "False" for all comparisons. This result indicates the absence of statistically significant differences between any of the compared sets.

The ANOVA analysis and the post-hoc Tukey test indicate that there are no significant differences between the means of the candidate material groups stored under ambient conditions, at 4°C and at -18°C.

Although the variability of the material differs between storage conditions, the means of the measured values are not sufficiently dispersed to conclude that one of the storage conditions has a significant effect on the material.

These results suggest that, statistically, all three storage conditions are similar in terms of their impact on the measured values of the material. However, the stability of the variability, reflected by the standard deviation, may provide additional useful information for the final decision regarding optimal storage conditions.

Chapter 8. Development of Breadcrumb Reference Material for Acrylamide Content

In the contemporary food industry, food processing is a standard practice, using technologies such as frying, baking and boiling to diversify tastes and textures and preservation methods like freeze-drying and pasteurization to improve the safety and quality of products. These processes optimize the flavor and appearance of foods, eliminate pathogenic microorganisms and extend shelf life, ensuring freshness and safety for consumption. However, thermal processing techniques can generate harmful chemical compounds, such as acrylamide, hydroxymethylfurfural (HMF) and furfural, which are not naturally present in food and can have mutagenic, carcinogenic and cytotoxic effects.

The main concern related to thermal treatments is the generation of acrylamide, a food toxin identified in high concentrations in food rich in asparagine and reducing sugars, exposed to temperatures above 120°C. This is associated with an increased risk of cancer and other health conditions. The European Food Safety Authority has recognized acrylamide as a major public health issue, recommending strict measures to reduce exposure to this compound. In 2017, the European Union issued regulations setting maximum allowable limits for acrylamide content in various food categories, thus promoting responsible practices in the food industry to protect consumers.

The selection of raw materials is a crucial step in creating a breadcrumb reference material for acrylamide content. The quality and chemical characteristics of the raw materials directly influence the formation of acrylamide and controlling variability between batches is crucial for the reproducibility of results.

Thus, different types of breadcrumbs were analyzed in a feasibility study for the development of the candidate reference material, with breadcrumbs being purchased commercially from various producers.

The breadcrumbs used as raw material must meet three vital criteria: they must have a uniform granulation, contain adequate levels of asparagine and reducing sugars and be completely free of contaminants and additives. These essential criteria significantly contribute to improving the homogeneity of the breadcrumbs used for developing the reference material. Following the feasibility study, it was decided to use breadcrumbs derived from bread made from type 650 white flour for the development of the candidate reference material.

The choice of breadcrumbs from type 650 white flour for the candidate reference material is justified by their fine granulation and uniform texture, thus ensuring a homogeneous distribution of properties in the final product. The moderate protein and gluten content of type 650 flour contributes to the uniform formation of acrylamide in the final product.

The Next Step Involves Applying Thermal Treatment to the Selected Breadcrumbs.

This process is essential for increasing and uniformizing the acrylamide levels, thereby facilitating the homogeneity of the reference material. Thermal treatment in the laboratory oven can affect the homogeneity of the breadcrumbs through structural and chemical changes. Uniform evaporation of moisture and adequate thermal distribution are crucial for maintaining consistency. However, variations in temperature and time can cause non-uniformities and

clumping, affecting density and texture. Therefore, rigorous control of thermal parameters is crucial for ensuring the integrity of the material.

During the thermal treatment of breadcrumbs (Figure 8.1), two major issues can arise: the upper layer may suffer from excessive drying, affecting quality and consistency, while the lower layer, in direct contact with the surface of the vessel, may lead to pyrolysis, causing variations in the material's composition. These differences in heat distribution can negatively influence the uniformity of the thermally treated breadcrumbs' characteristics.

Thus, it is essential to consider parameters such as the type of container used and the uniform distribution of breadcrumbs within it, to reduce the heterogeneity effects generated by contact with the metal surface. A careful approach to controlling thermal treatment parameters, such as temperature and exposure duration, is crucial to ensure the consistency and homogeneity of the material.

To disperse any clumping of breadcrumb particles caused by water evaporation following thermal treatment, an additional sifting operation was performed using a single sieve. This additional sifting procedure was conducted after the thermal treatment of the breadcrumbs to break up clumps and uniformize the material's granulation, thereby improving its quality and homogeneity.

Fig. 8.1 Thermal Treatment of Raw Material

The raw material was distributed to a thickness of 15 mm on the surface of a stainless steel tray. A stainless steel tray was chosen due to its superior thermal conductivity properties, which allow for uniform heat distribution across the tray surface. After the treatment, the breadcrumbs were packed and labeled in polypropylene Falcon tubes (Figure 8.2).

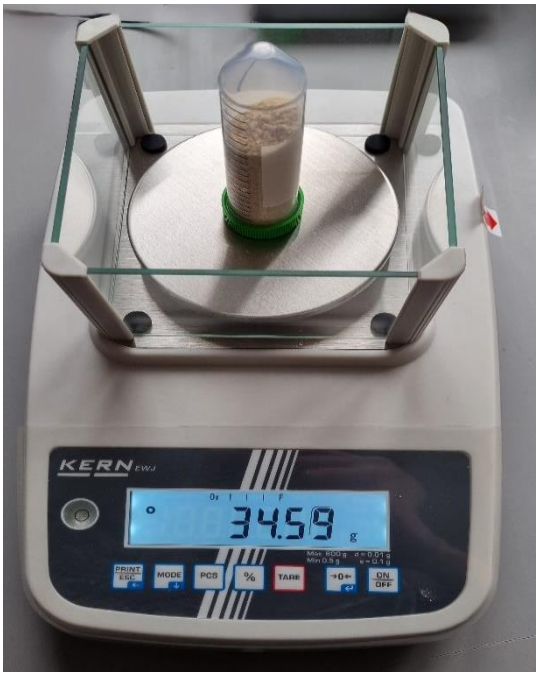

Fig. 8.2 Candidate Reference Material
Packaged in PP Falcon Tube

Thus, four experimental variants were created, as presented in Table 8.1, with the aim of evaluating the formation and establishing the optimal level of acrylamide in the breadcrumbs.

Table 8.1. Experimental Variants Used in This Study

Experimental variant	**V1**	**V2**	**V3**	**V4**
Breadcrumb Batch	L1	L1	L1	L1
Breadcrumb Processing Conditions				
Frying Temperature (°C)	170			
Frying Duration (minutes)	120	120	180	300
Breadcrumb Layer Thickness (mm)	15			
Breadcrumb Granulation (D), µm	355 < D < 1000	D< 355	355 < D < 1000	D< 355

The breadcrumbs were subjected to a frying process at a constant temperature of 170°C, with variable durations: 120 minutes for variants V1 and V2, 180 minutes for variant V3 and 300 minutes for variant V4. The differences induced by the thermal treatment are evident, as presented in Figure 8.3, illustrating the variability in the appearance of the breadcrumbs based on the experimental conditions applied to each variant.

V1 (170°C, 120 minutes, 355 µm < D < 1000 µm)

V2 (170°C, 120 minutes, D < 355 µm)

V3 (170°C, 180 minutes, 355 µm < D < 1000 µm)

V4 (170°C, 300 minutes, D < 355 µm)

Fig. 8.3 Differences Induced by Thermal Treatment

The moisture content of the breadcrumbs is essential for their quality and durability, directly influencing the drying process at 170°C, which prevents the development of microorganisms and extends shelf life. Careful management of the drying process ensures uniformity and consistency of the final product, preventing quality issues such as uneven textures or premature deterioration. Optimal moisture levels play a crucial role in sample preparation and analysis using chromatographic techniques, such as HPLC and GC, affecting the efficiency and precision of analytical determinations.

Inadequate moisture levels can negatively impact the extraction of acrylamide from breadcrumbs, affecting the reproducibility and comparability of analytical results. Excessive moisture dilutes the analyte, complicating its separation, while too little moisture hinders the complete dissolution of the analyte in the extraction solvent. Rigorous control of moisture, standardization of samples and adjustment of analytical methodologies are essential for obtaining precise and reproducible results, thus ensuring the quality of analytical data in acrylamide determination.

To determine the moisture content of the raw material used for producing the reference material for breadcrumb matrix, the reference method described in ISO 712:2009 was used. Units of the candidate reference material were randomly sampled and analyzed in triplicate. The results obtained from the moisture content analysis of the samples are presented in Table 8.2:

Table 8.2 Moisture Content for Candidate Reference Material from Breadcrumbs

Variant 1		Variant 2		Variant 3		Variant 4	
Sample	D.M%	Sample	D.M%	Sample	D.M%	Sample	D.M%
P1	99.28	P4	98.93	P7	98.70	P10	99.58
P1	99.25	P4	99.00	P7	98.71	P10	99.50
P2	99.27	P5	98.96	P8	98.81	P11	99.69
P2	99.17	P5	98.89	P8	98.87	P11	99.61
P3	99.13	P6	98.80	P9	98.96	P12	99.73
P3	99.30	P6	98.92	P9	98.92	P12	99.75

The graphic in Figure 8.4 provides a precise visualization of the average dry mass content (S.D.%) for each breadcrumb variant, using mean points and 95% confidence intervals. The results indicate that Variant 4 has the highest average moisture content, while Variant 3 exhibits the lowest average values.

Variant 1 shows the least variability, indicating a lower inconsistency in moisture levels across its samples. Significant differences between confidence intervals suggest substantial variations between variants, which impact the physical and chemical properties of the breadcrumbs.

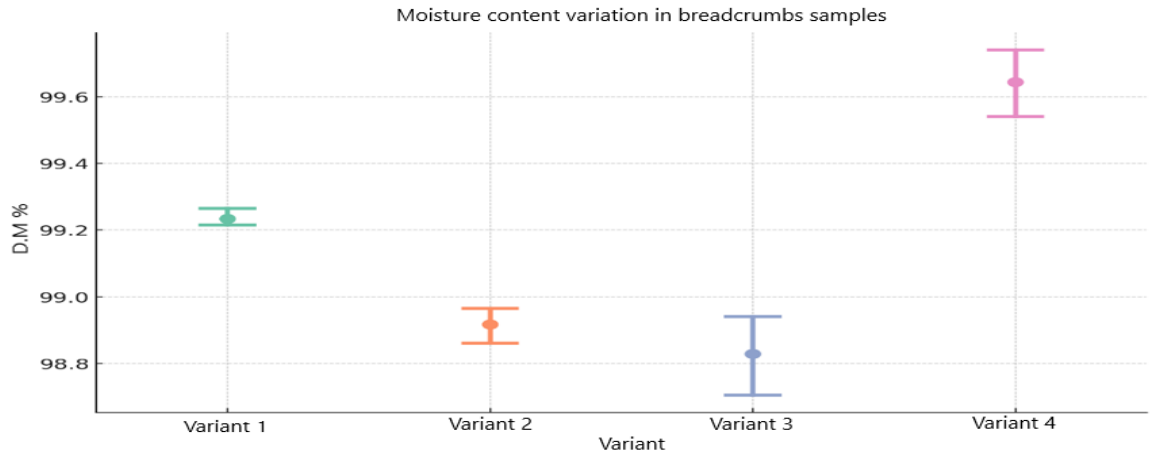

Fig. 8.4 Variation in Moisture Content in Breadcrumb Samples

To determine the acrylamide content in the candidate reference material, a method based on water extraction of acrylamide (AA) from the matrix under study was used. The extract is centrifuged and the supernatant is purified using two solid-phase extraction (SPE) columns.

The purified AA extract is then derivatized with bromine compounds (potassium bromide, hydrobromic acid and bromine water) to produce the dibrominated AA derivative

(2,3-DBPA). The dibrominated AA derivative (2,3-DBPA) is extracted from the derivatized solution with a mixture of ethyl acetate and hexane (4:1, v/v), followed by solvent evaporation and reconstitution of the extract in ethyl acetate and triethylamine. Triethylamine is added to convert the dibrominated AA derivative (2,3-DBPA) into a more stable monobrominated AA derivative (2-BPA) for GC analysis. The obtained extract is filtered through a regenerated cellulose microfilter with 0.2 µm pores (Spartan 13RC) and 17 mm diameter and then 1 µL is injected into the chromatograph.

According to Regulation 2158/2017, samples from the experimental variants (bread-based breadcrumbs) fall into the category "Products similar to other products in this category" with a reference level of 300 µg/kg.

The "BREADCRUMBS" product, manufactured in the 4 experimental variants, showed values approximately 5 – 8 times higher (1,499.25 - 2,421.44 µg/kg) compared to the reference level of 300 µg/kg set for this product category.

Table 8.3 Acrylamide Levels in Breadcrumb Samples from Experimental Variants

VARIANT	Flask	Replicate	Average, µg/kg	Average ± SD, µg/kg	RSD (r), %
V1 (170°C, 120 minutes, 355 µm < D < 1000 µm)	F11	P1	1.503,68	1.509,12 ± 7,68[a]	0,51
		P2	1.514,55		
	F12	P1	1.484,12	1.484,71 ± 0,82[a]	0,06
		P2	1.485,29		
	F13	P1	1.497,89	1.503,94 ± 8,55[a]	0,57
		P2	1.509,98		
	AVERAGE V1 (n = 6)			**1.499,25 ± 11,49**	**0,77**
V2 (170°C, 120 minutes, D < 355 µm)	F21	P1	2.281,64	2.278,50 ± 4,44[b]	0,19
		P2	2.275,36		
	F22	P1	2.467,72	2.504,17 ± 51,56[a]	2,06
		P2	2.540,63		
	F23	P1	2.468,99	2.481,66 ± 17,91[a]	0,72
		P2	2.494,33		
	AVERAGE V2 (n= 6)			**2.421,44 ± 124,31**	**5,13**
V3 (170°C, 180 minutes, 355 µm < D < 1000 µm)	F31	P1	1.869,51	1.820,54 ± 69,25[a]	3,80
		P2	1.771,57		
	F32	P1	1,745,90	1.806,76 ± 86,06[a]	4,76
		P2	1,867,61		
	F33	P1	1.776,36	1.773,93 ± 3,44[a]	0,19
		P2	1.771,49		
	AVERAGE V3 (n= 6)			**1.800,41 ± 23,95**	**1,33**
V4 (170°C, 300 minutes, D < 355 µm)	F41	P1	1.893,92	1.883,55 ± 14,66[b]	0,78
		P2	1.873,18		
	F42	P1	1.963,47	1.964,96 ± 2,11[b]	0,11
		P2	1.966,46		
	F43	P1	2.126,17	2.094,19 ± 45,22[a]	2,16
		P2	2.062,22		
	AVERAGE V4 (n= 6)			**1.980,90 ± 106,22**	**5,36**

*Values that do not share the same letter within the same variant are significantly different.

For each of the three flasks of an experimental variant, two parallel determinations were performed (4 variants × 3 flasks × 2 determinations). The arithmetic mean for the two parallel determinations was calculated, meeting the repeatability condition.
Samples from V1 and V2 come from the same batch of breadcrumbs and were produced under the same frying conditions (170°C, 120 minutes). Samples from V2 (sieved) resulted from sifting the fried breadcrumbs, which had a finer granulation, D < 355 μm, whereas V1, with larger granulation (355 μm < D < 1000 μm), was the reject.

It was observed that in V2, where the breadcrumb granulation was finer, the acrylamide (AA) level was higher, 2,421.44 ± 124.31 μg/kg, compared to V1, which had a higher particle size and an AA level of 1,499.25 ± 11.49 μg/kg. The finer granulation in V2 led to better AA extraction compared to V1.

Despite efficient AA extraction in V2, where particle sizes were sufficiently small and SPE purification was better, cleaner extracts were obtained compared to V1 (see Figures 10 and 13), which had larger particle sizes.

The same pattern was observed for variants V3 and V4. The AA level was higher in V4 (1980.90 ± 11.49 μg/kg) where particle size was finer (<355 μm), compared to V3 (1800.41 ± 23.95 μg/kg), where particle size was larger (355 μm < D < 1000 μm). The higher AA level in V4 was also due to a longer frying duration of the breadcrumbs.

Thus, samples from variants (V2, V4) with finer particle sizes (<355 μm) resulted in better extraction and purification compared to samples from variants (V1, V3) with larger particle sizes (355 μm < D < 1000 μm).

Measurement variability was indicated by the coefficient of variation (CV) or relative standard deviation (RSD). This was better (0.77% and 1.33%) in V1 and V3, where the granulation was larger, compared to 5.13% and 5.36% in variants V2 and V4, where the granulation was smaller.

Homogeneity of samples from the three flasks of the same variant was also tested using ANOVA with the Tukey method. The analysis revealed that there were no significant differences between flasks in variants V1 and V3. However, in variants V2 and V4, significant differences were found between F21 and F22, F23 and between F42, F41 and F43 ($p < 0.05$).

Chapter 9: Final Conclusions and Main Contributions to “Optimizing Quality Management in Agro-Food Product Testing”

Chapter 9 presents the final conclusions and main contributions of the doctoral thesis on optimizing quality management in agro-food product testing. The analysis of the current state, detailed in Chapter 3, led to essential conclusions and outlined relevant research and development directions. The primary objective of the research was to develop and implement a quality management system in accordance with ISO 17034, aiming to optimize the processes necessary for developing reference materials. Implementing this system ensures compliance with international requirements for competence and quality, integrating the principles of the standard into all stages of production and control.

Studies in Chapter 5 described laboratory-scale production activities for cereal reference materials according to ISO 17034 and ISO Guide 35, evaluating homogeneity and stability of the reference materials. Chapter 6 detailed the structure and implementation of the stability study for reference material MR001-IBA, in accordance with ISO 17034:2017. Chapter 7 summarized activities for mitigating identified risk factors using techniques such as HTA and SWOT. Chapter 8 described the experimental development of a new reference material from breadcrumbs for acrylamide content determination.

In achieving the primary goal of the doctoral work, the thesis provides significant contributions, including: a critical analysis of the current state of knowledge, documentation of requirements for developing a quality management system according to ISO 17034, selection of suitable raw materials, production of reference material batches, detailed experimentation to assess their quality and initiation of the development of an acrylamide reference material. These achievements demonstrate the laboratory's capability to produce high-quality reference materials, essential for standardizing and calibrating analytical methods in the agro-food sector.

Bibliografie Selectivă

[1] A. S. Floarea SERBANCEA, Nastasia BELC, Ovidiu MARCULESCU and A. STANESCU, "THE DEVELOPMENT OF QUALITY MANAGEMENT SYSTEM USED IN THE PRODUCTION OF REFERENCE MATERIALS," *Proceedings of the 7 th Review of Management and Economic Engineering International Management Conference*, 2020.

[2] A. S. Floarea SERBANCEA, Nastasia BELC, Ovidiu MARCULESCU and V. L. Aurelia STANESCU, "THE ROLE OF EUROPEAN UNION POLICIES ON THE MANAGEMENT OF REFERENCE MATERIALS SPECIFIC TO FOOD ENGINEERING," *Proceedings of the 7 th Review of Management and Economic Engineering International Management Conference*, 2020.

[3] O. I. pentru Standardizare, "ISO/CEI 17000:2004 Evaluarea conformității. Vocabular și principii generale," 2004.

[4] International Organization for Standardization, "ISO 17034:2016 General requirements for the competence of reference material producers," 1. [Online]. Available: https://www.iso.org/standard/29357.html

[5] "Mape :: RENAR." Accessed: Jun. 20, 2024. [Online]. Available: https://www.renar.ro/index.php/acreditarea/procesul-de-acreditare/documente-pentru-acreditare/mape

[6] T. S. Picker, “Digitalization in Laboratories of the Pharmaceutical Industry,” *Solid State Development and Processing of Pharmaceutical Molecules: Salts, Cocrystals and Polymorphism: Volume 79*, vol. 79, pp. 397–420, Jan. 2021, doi: 10.1002/9783527823048.CH8.

[7] F. Șerbancea, O. Marculescu, F. Nenciu and A. Stanescu, “Feasibility study regarding the production of the reference material RM001F- IBA wheat flour.,” in *International Symposium, Isb-inma Teh’, Agricultural And Mechanical Engineering, Bucharest, Romania, 29 October 2021. Proceedings*, Bucharest: National Institute of Research – Development for Machines and Installations Designed for Agriculture and Food Industry – INMA Bucharest, 2021, pp. 216–223.

[8] O. MARCULESCU, N. BELC, R.-M. MARINESCU, C. SERBANCEA and A. SEMENESCU, “THE INFLUENCE OF FOOD MATRIX IN THE DEVELOPMENT OF REFERENCE MATERIALS,” *SCIENTIFIC PAPERS-SERIES D-ANIMAL SCIENCE*, vol. 65, no. 1, pp. 511–516.

[9] O. MARCULESCU, C. SERBANCEA, E. C. GRADEA and A. SEMENESCU, “THE INFLUENCE OF TEMPERATURE ON THE STABILITY OF REFERENCE MATERIALS,” *SCIENTIFIC PAPERS-SERIES D-ANIMAL SCIENCE*, vol. 65, no. 1, pp. 505–510, 2022.

[10] A. Culeţu, M. Mulţescu, … I. S.-A. of the F. of and undefined 2023, “SELECTION OF THE TYPE OF WHEAT FLOUR IN THE DEVELOPMENT OF A REFERENCE MATERIAL FOR THE ANALYSIS OF THE WET GLUTEN,” *annals.fih.upt.ro*, Accessed: Jun. 10, 2024. [Online]. Available: https://annals.fih.upt.ro/pdf-full/2023/ANNALS-2023-2-22.pdf