Scientific Papers. Series D. Animal Science. Vol. LXV, No. 1, 2022
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

# THE INFLUENCE OF TEMPERATURE ON THE STABILITY OF REFERENCE MATERIALS

**Ovidiu MĂRCULESCU [1,2], Cristian ȘERBANCEA[2], Elena Claudia GRADEA[2], Augustin SEMENESCU[1,3]**

[1]University Politehnica of Bucharest, 313 Splaiul Independentei, 060032, Bucharest, Romania
[2]National Research and Development Institute for Food Bioresources – IBA Bucharest, 5th Ancuta Baneasa Str., 2nd District, Bucharest, 020323, Romania
[3]Academy of Romanian Scientists, 3 Ilfov, 050044, Bucharest, Romania

Corresponding author email: marculescu.ovidiu90@gmail.com

***Abstract***

*The paper presents the influence of temperature on the stability of the reference material, wheat flour matrix, MR001F-IBA. Experimentally, the stability of the candidate reference material units MR001F-IBA were evaluated in different storage conditions using a temperature of 4°C and, respectively, a range between 25-30°C degrees. The 3 batches of candidate reference material evaluated were produced according to the requirements of the ISO 17034: 2017 "General requirements for the competence of reference material producers". The results obtained by testing the MR001F-IBA reference material units according to the ISO 2171: 2007 standard indicate that there are no differences in the ash content in the analysed samples. The results are interpreted statistically according to the recommendations from the ISO 35: 2017 guide, these substantiating the information necessary for the MR001F - IBA certification. The purpose of the paper was to establish the conditions in storage, transport of MR001F-IBA for ash content.*

***Key words**: ash content, reference material, wheat flour, stability.*

## INTRODUCTION

The agri-food system has developed rapidly in recent decades due to the technology that has allowed the rapid transportation of products globally. Along with the agri-food sector development, many problems related to transport, storage and most importantly food safety have surfaced.

Because food safety plays a pivotal role in our current society the necessity of improving the monitoring of agri-food products and enhancing the quality of measurements in laboratory testing had become a major concern across member states of the European Union.

To support food safety on the ongrowing agri-food market, the European Union imposed regulations and developed programs to support the fight against food fraud.

The projects, under the guidance of the E.U., had supported and encouraged the development and production of reference materials which are essential for calibration, method validation, estimation of measurement uncertainty, for quality assurance, quality control and to ensure trasability.

After analysing specific databases and the catalogues of companies which are reference materials producers, it was noticed that there is a small number of reference materials regarding the wheat flour.

Wheat flour is an essential component in human nutrition, and it is being produced in huge quantities around the globe.

The European Union is being ranked as the second largest consumer which strongly suggests that there is a need to develop a reference material, with specific parameters for testing the quality of wheat flour.

Ash content is one of the major indicators for the quality and use of flour, which is essentially a characteristic that determines the purity of wheat flour. The ash obtained from flour consists of mineral compounds of phosphorus, potassium, calcium, magnesium, iron, zinc and copper, being a source of concentrated and insoluble fibre (Czaja et al., 2020).

Indirectly, ash is an indicator of mill performance as it shows the degree of contamination of bran flour, which represents the outer layer of wheat grain consisting of pericarp and aleurone.

For most baking applications, bakers are looking for excellent quality flour with high protein levels and the highest purity in terms of endosperm content (Carson & Edwards, 2009).
Ash is a measure of mineral content and is used to grade flour into different varieties. For example, whole wheat flour has a higher ash level than white flour. By quantifying ash levels during processing, flour millers can maximize extraction efficiencies and optimize blending (https://assets.thermofisher.com/TFS-Assets/MSD/Application-Notes/AN52269-wheat-flour-protein-moisture-ash-analysis-ft-nir.pdf.)
The determination of the amount of ash contained in wheat flour is necessary for its classification (Table 1).

Table 1. Types of wheat flour

| Type of wheat flour | Ash content % (max) | Application |
|---|---|---|
| Type 000 | 0.48 | Used for puffs and light pastries. It is a fine flour with a low protein content, about 9%. |
| Type 480 | 0.48 | It is also used for the preparation of pastries. |
| Type 550 | 0.55 | A harder flour than the first two, usually used for almost any kind of dough. |
| Type 650 | 0.65 | It has a higher gluten content which makes it used for doughs that need leavening(fermentation). It has a high protein content of 12-14%. |
| Type 1250 | 1.25 | Brown bread contains the largest proportion, over 50%, of wholemeal wheat flour type 1250. |
| Type 1350 | 1.35 | Brown bread containing the highest proportion, over 50%, wholemeal wheat flour type 1350. |
| Type 1750 | 1.75 | Contains wheat graham flour, meaning wholemeal or dietary flour type 1750 with the addition of wheat bran. |

## MATERIALS AND METHODS

In the ISO 17034: 2016 "General requirements for the competence of reference material producers" the reference material is defined as a sufficiently homogeneous and stable material, with one or more assigned properties, considered suitable for the intended use (https://www.iso.org/standard/29357.html).
Therefore, the definition shows that homogeneity and stability are two characteristics that play an important role in the development and production of reference materials.
The assessment of stability for a reference material implies that the stability should be assessed for each assigned property to determine the risk factors to which it may have been exposed, like temperature, humidity, or light, that may influence or degrade these properties over a predetermined time period.
ISO Guide 35: 2017 "Reference materials - Guidance for characterization and assessment of homogeneity and stability" approaches in greater detail the assessment of the stability of the reference material.
The guide ISO 35: 2017 describes two types of stability that are relevant when a reference material is produced. The long-term stability which is corelated with the behaviour of RM in the producer's facility when it is stored under the specified conditions. The second type of stability described is the short-term stability or transport stability which requires testing under the reasonable transport condition (https://www.iso.org/ standard/60281.html).
The stability of the reference material is determined by analysing the values of the certified parameters in the samples of units stored at the recommended temperatures for the reference material, assuming that there is no change in the composition of the reference material at this temperature (Rutkowska et al., 2020)
The candidate reference material used in the experimental stability study was produced within the National Research and Development Institute for Food Bioresources - IBA Bucharest, which followed the production process according to ISO 17034: 2017 meaning that the same raw material was used, under the same conditions, following the same process. A first step in the production process of the candidate reference material involves homogenization, which is made by using the electromagnetic sieving equipment BA 300 N, for-which a single sieve, certified ISO 3310, was used with a ⌀ of 1 mm.
The sample divider Retch PT100 with the vibrating splitter DR100 is used for portioning

and packaging. The sample divider distributes equal fractions of the base material to each unit of the candidate reference material, which can be considered an additional homogenisation.
The units of candidate reference material are sealed with an aluminium foil, preventing moisture transfer, using an electromagnetic induction equipment with forced cooling to avoid the risk of degrading the matrix by exposing it to high temperatures.
Three batches were produced, the first one was used in the feasibility study, the other two, presented in this paper, were used to assess the homogeneity and stability for the reference material.

## RESULTS AND DISCUSSIONS

The ash content for the wheat flour used in the production of MRC, was determined by using the method described in ISO 2171: 2007 “Cereals, pulses and by-products - Determination of ash yield by incineration” (https://www.iso.org/standard/37264.html).
For applying the method described in ISO 2171: 2007 to determine the ash content of wheat flour it is necessary to prepare the crucibles suitable for use at 550°C by drying them in an oven at a temperature of 130°C for a period of 90 minutes. After drying, the crucibles are removed from the oven and placed in the desiccator to cool, then weighed with the precision of 0.1 g. The preparation of the working sample is carried out by weighing between 4.9 g to 5.1 g of sample for incineration at 550°C. It is permitted to use between 2 g and 3 g sample for low density products. The sample must be evenly distributed without being compacted in the crucible.
After preparing the sample, the crucible is placed at the entrance to the furnace, for the sample to reach the incineration temperature for combustion, ethanol was added, as is it is permitted in the case of incineration at 550°C (Figure 1).
When the sample has finished burning it should be placed in the oven for a period of at least 4 hours.

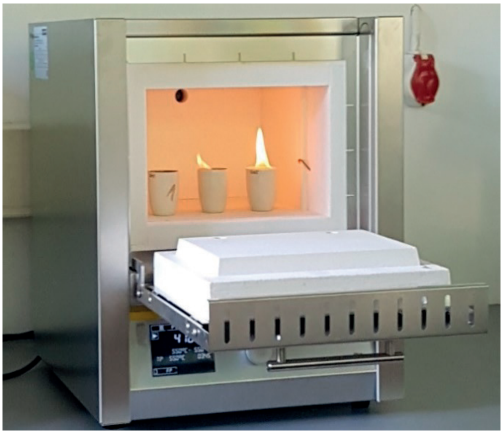

Figure 1. Sample combustion in the furnace

When the incineration is complete, the crucibles are removed from the oven and quickly placed in the desiccator for cooling, between 60 to 90 minutes (Figure 2).
It must be recalled that ash has a high degree of water absorption. Therefore, as soon as the samples reach room temperature, they must be weighed with a precision 0.1 g.

Figure 2. Incinerated sapless in the desiccator

The results obtained following the analysis of the candidate reference material units on the ash content of wheat flour, using the method described in ISO 2171: 2007 for each point in time, are presented in Table 2.

Table 2 Results obtained following the analysis of the candidate reference material units

| Crt. | T0 | | | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|---|---|
| Sample | 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 |
| Average | 0.53 | 0.51 | 0.54 | 0.54 | 0.52 | 0.53 | 0.54 |
| Standard deviation | 0.026 | 0.012 | 0.015 | 0.014 | 0.015 | 0.025 | 0.017 |

A one-way ANOVA was performed to compare the ash content from the analysed units from which it can be observed that there are no significant differences between the averages of the analysed groups. The results are presented in Table 3.

Table 3. One-way ANOVA results for the second batch

| | DF | Sum of Square | Mean Square | F Statistic | P-value |
|---|---|---|---|---|---|
| Group | 6 | 0.002362 | 0.000394 | 1.117 | 0.401041 |
| Residual | 14 | 0.004933 | 0.000352 | NaN | NaN |
| Total | 20 | 0.007295 | 0.000746 | | |

Since the p-value is 0.4010, being higher than the confidence level ($\alpha = 0.05$), and F-statistic does not exceed the critical value, we cannot reject the null hypothesis, which states that the averages of the 7 candidate reference material units analysed are equal, as we can observe re in figure 3.

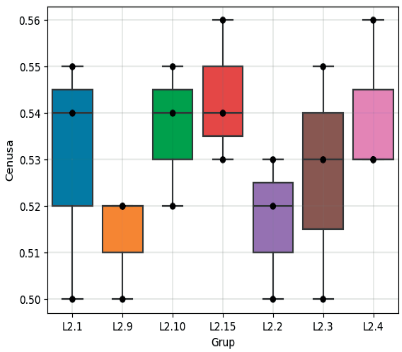


Figure 3. Graphic representation of ANOVA results

The third batch was produced from the same raw material as the first two batches. From the specialised literature and the results obtained from the first two batches, which were analised and provided enough data, that it can be presumed that the ash content is sufficently homogeneus and stable across every batch produced (Figure 4).

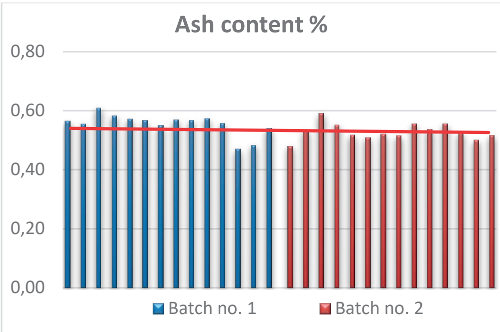


Figure 4. Ash content result for first and second batch

It was decided that for this lot the interval at which the ash content is determined to increase. In order to determine the optimal storage conditions for the candidate reference material, the third batch was randomly divided into two groups to be stored under different environmental conditions.

The first group being stored at a temperature of 4°C, and the second group at a temperature ranging between 25°C and 30°C.

Table 4 shows the results obtained at $T_0$ and $T_1$ for the ash content in the third batch.

For the interpretation of the data, the analysis of the variance was also chosen to verify if there are significant differences between the 4 samples.

Table 4. Results obtained at T0 and T1 for the ash content in the third batch

| Crt. | T0 | | T1 | |
|---|---|---|---|---|
| Sample | L3.10H | L3.2F | L3.5H | 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 |
| Average | 0.51 | 0.51 | 0.55 | 0.52 |
| Standard Deviation | 0.0110 | 0.0204 | 0.01221 | 0.0168 |
| *H – temperature range between 25 – 30°C<br>*F – stable temperature 4°C | | | | |

After examining the results obtained from the analysis of variance it can be observed that the value of p is higher than α, and statistic F does not exceed the value of critical F, which is 3.376 (Table 5).

Table 5. One-way ANOVA results for the 3rd batch

| | DF | Sum of Square | Mean Square | F - value | P-value |
|---|---|---|---|---|---|
| Group (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 | | |

The data shows that the null hypothesis cannot be rejected. This means that there are no significant differences between the averages of the 4 groups analysed.

Figure 5 shows graphically the results of the analysis of variance for the 3rd batch on ash content.

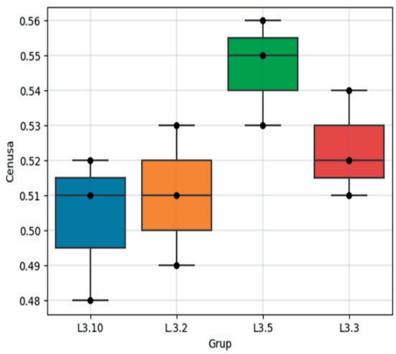


Figure 5. Graphic representation of ANOVA results for the 3rd batch

## CONCLUSIONS

This study has shown that values of the ash content do not vary within and between batches produced from the same raw material.

The research has also shown that the temperature does not influence the analyte studied in the wheat flour matrix under the conditions of the presented experiment. In this context, both the storage and the transport of MR001F - IBA can be done in the temperature range 4°C - 30°C, which leads to the reduction of energy consumption in storage and transport. The study also provides the information needed for MR001F-IBA certification.


## ACKNOWLEDGEMENTS

This paper was supported by a grant offered by the Romanian Minister of Research, Innovation and Digitalization, as Intermediate Body for the Competitiveness Operational Program 2014-2020, call POC/78/1/2/, project number SMIS2014 + 136213, acronym METROFOOD-RO and Romanian Minister of Research, Innovation and Digitalization, as Core Program, contract 22N/2019, project PN 19 02 04 01 "Research on the development of skills in the development of reference materials and inter laboratory comparisons".


## REFERENCES


Czaja, T., Sobota, A., & Szostak, R. (2020). Quantification of ash and moisture in wheat flour by Raman spectroscopy, *Foods*, 9(3), doi: 10.3390/FOODS9030280.

Carson, G. R., & Edwards, N. M. (2009). CHAPTER 4: Criteria of Wheat and Flour Quality, *WHEAT Chem. Technol.*, 97–118, doi: 10.1094/9781891127557.004.

Fast and reliable quality analysis on wheat flour protein, moisture, and ash values using FT-NIR. https://assets.thermofisher.com/TFS-Assets/MSD/Application-Notes/AN52269-wheat-flour-protein-moisture-ash-analysis-ft-nir.pdf (accessed Nov. 04, 2022).

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

International Organization for Standardization, "ISO Guide 35: 2017 (2017). Reference materials - guidance for characterization and assessment of homogeneity and stability," *4*. p. 105, [Online]. Available: https://www.iso.org/standard/60281.html.

Rutkowska, M., Namieśnik, J., & Konieczka, P. (2020). Production of certified sreference materials - homogeneity and stability study based on the determination of total mercury and methylmercury. *Microchem. J.*, 153, doi: 10.1016/j.microc.2019.104338.

ISO 2171:2007. Cereals, pulses and by-products — Determination of ash yield by incineration. https://www.iso.org/standard/37264.html (accessed Apr. 11, 2022).