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THE INFLUENCE OF FOOD MATRIX IN THE DEVELOPMENT OF REFERENCE MATERIALS

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Abstract

The paper presents the influence of the raw material (matrix) in the homogeneity and stability assessment of two batches of candidate reference material MR001F - IBA produced at an interval of 30 days and evaluated for three months. The statistical interpretation of the moisture content of wheat flour type 650 indicates the need to carry out feasibility studies for each batch made under the same processing conditions. The feasibility study carried out followed the implementation of the recommendations from the ISO 35: 2017 guide regarding the risk in the stability of the reference material produced in successive batches. Stability has been assessed according to section 8.3.2.1 - Classical stability studies - Repeatability conditions of measurement from the ISO 35:2017 guide.

Key words: *homogeneity, reference material, stability, wheat flour.*

INTRODUCTION

The most developed industry, at European level, is the agri-food industry, with cereal processing being the most important sector. The annual amount of produced flour is around 35 million tons of flour, obtained through processing over 50 million tons of wheat, rye and oats.

Among cereals, wheat has the potential to contain the highest quantity of gluten, which is what creates the elasticity and strength of the dough and influences the texture of pastries.

It has been shown that the temperature in storage affects the qualities attributed to wheat flour. Unfortunately, only a few studies have been conducted to evaluate the influence of storage conditions on the properties of flour (Sujitha et al., 2018).

Wheat flour is the most important product in the milling industry, its quality can be defined as the set of organoleptic, physical, chemical and rheological characteristics imposed by the processing requirements for the purpose for which it is intended.

The purpose of flour is different depending on the industry in which it will be processed: bread and bakery products, pasta, etc. (Dabija, 2016).

The importance of the quality of wheat flour is due to the fact that it is a biological material that can be obtained from different sources that can vary significantly and can influence its characteristics.

The quality of wheat flour is closely related to moisture, having a special importance since a lower moisture content means a higher number of solid components (protein, starch, fat, sugar, ash) speculated in the bakery industry but also due to the fact that high humidity is a risk factor for microbial and fungal development and has a significant effect on protein and raw fats. However, it has been observed that it does not have a significant effect on the fiber and ash content (Nasir et al., 2003).

The moisture content of wheat flour is also of particular importance in determining the shelf life: the lower the moisture content, the longer the shelf life.

Appropriate methods and procedures for laboratory testing need to be used for quality control and assurance, as this sector of the agri-food industry is a key point in food safety.
Given that food safety is a major priority for the European Union (EU), it has invested research programs to improve the quality of analytical measurements (physical, chemical and biological) by developing reference materials (Maier et al., 1997).
The role of reference materials in the quality of analytical results in laboratory testing has received increasing attention in recent years, especially in the agri-food industry where they are being used for method validation, calibration, QA and QC, trasability.
The quality of measurements have an important role in technological and socio-economic development, thus supporting the development of trade and monitoring the quality of products and services and allows the optimization of decisions in monitoring food safety (Rychlik et al., 2018).
Reference materials are defined as sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process (International Organization for Standardization, 2016). The definition highlights two important characteristics of a reference material: homogeneity and stability.

MATERIALS AND METHODS

The production of a reference material requires, according to standard 17034:2017 (Standardization, 2016), an experimental stability study to determine whether the value of the properties for which the candidate reference material was characterized varies or degrades due to the environmental conditions under which was exposed. Conditions such as: temperature, humidity and light.
In order to test the stability of a reference material, sampling units are randomly extracted, an exception being made at an early stage in the development of a reference material, when it is possible to produce experimental batches of small size, subjected to treatment processes, when necessary and packaging, which may be used in whole or part for stability testing.
The stability of the reference material is determined by analyzing 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 experimental stability study was performed on two batches of the candidate reference material produced within the National Research and Development Institute for Food Bioresources - IBA Bucharest, from the same raw material, under the same conditions, following the same process of production. Given that wheat flour is a sufficiently homogeneous natural matrix and that the parameter selected for the characterization of CRM obtained, moisture, respectively, the processing requires only homogenization and packaging under normal conditions (without inert gas).
For the preparation of a batch of candidate reference material, the homogenization was done using digital electromagnetic sieving equipment, with a single sieve, certified ISO 3310, while ensuring that the grain of wheat flour complies with ISO 712: 2009.
Packaging and portioning MR001F - IBA is performed using the Retsch Sample Divider

Figure 1. MR001F - IBA Candidate reference material

PT100 with vibrating splitter DR 100. Due to the fact that the device divides the sample so that the composition of each fraction of the sample corresponds exactly to that of the used material, an additional homogenization is being performed. The sealing of the vial was through electromagnetic induction and forced cooling using the automatic equipment LX6000A to eliminate the risk of heating of the matix.

The finished product was packaged in 250 g or 500 g bottles. After that, it is stored under predetermined storage conditions. It will be tested in experimental studies of homogeneity and stability according to ISO 17034:2009.

Figure 2. Batch produced for feasibility study

The moisture content of wheat flour used to produce the candidate reference material MR001F - IBA Wheat Flour was determined by using the reference method described in ISO 712:2009. Candidate reference material units were randomly selected and sampled for triplicate analysis. Approximately 5 g of type 650 flour with an accuracy of 0.001 g were weighed for each sample and placed in a metal capsule with lid, dried and weighed beforehand. After weighing, the capsules are placed in the oven at 130° C for 90 minutes.

Figure 3. Samples inside oven.

After 90 minutes, the capsules are removed and placed in the desiccator for 30-45 minutes for cooling, followed by the final weighing using the analytical balance.

Humidity is calculated as a percentage using the formula $W_{H2O} = \left(1 - \frac{m_1}{m_0}\right) \times 100$, where m_0 is the mass of the working sample, and m_1 is the mass of the working sample after drying.

Figure 4. Final weighing of the candidate reference material

RESEARCH AND DISCUSSIONS

Using the method described above, six samples from the first batch of candidate reference material were analysed. The first three unites of candidate reference material MR001F - IBA Wheat Flour, were randomly selected when the first batch was produced, for the initial analysis (also called T_0). They were included in the homogeneity study; the rest of the samples have been-analysed at one-month interval.

The experimental study design used is a classical stability study described in ISO Guide 35:2017 (International Organization for Standardization, 2017).

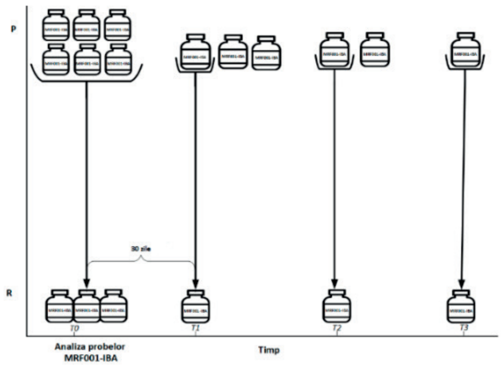

Figure 5. Representation of a classical stability study

Assessing moisture content on the first batch

The results of the experimental stability study for the first batch are presented in Table 1.

To determine whether there are significant differences in humidity between the six units of analyzed CRM, a one-way analysis of variance was applied. The results are presented in Table 2.

Table 1. Moisture content results for the first batch

Crt.	T0			T1	T2	T3
Sample	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
Average	10.14	10.13	10.15	10.21	10.24	10.20
SD	0.025	0.015	0.021	0.031	0.025	0.021

Table 2. One-way ANOVA result for the first batch

	DF	Sum of Square	Mean Square	F	P-value
Group	5	0.026711	0.005342	9.713131	0.000671
Residual	12	0.0066	0.00055	NaN	NaN
Total	17	0.333133	0.0019595		

The results indicate a statistically significant difference in humidity averages in the analyzed CRM samples. From Table 2 we can observe that F-value exceeds the value of F critical, and the p value is 0.000671, which means that p is less than the significance level α which is equal to 0.05, indicating a confidence level of 95%.

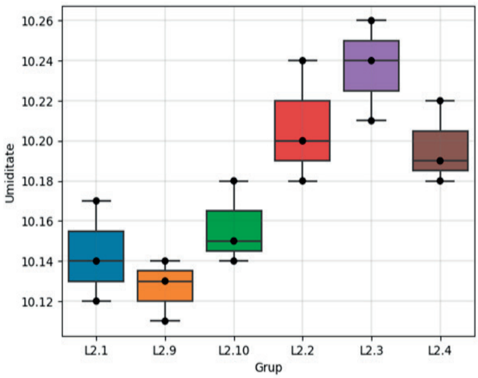

Figure 6. ANOVA chart for the first

Thus, it can be deduced that the null hypothesis is rejected, resulting in the fact that there are significant differences between the averages of the 6 groups, presented in chart (Figure 6).

In order to determine exactly where the differences are located in the ANOVA analysis, it was performed a post-hoc test, called Tukey HSD, which is a multiple comparison procedure, also called the significantly honest difference test.

Table 3 shows the values resulting from the application of the Tukey HSD test, which shows that there are significant differences between certain groups.

Table 3. Tukey HDS results

Group 1	Group 2	Mean Diff	Crit Diff	P-adj	Reject
L2.1	L2.10	0.0133	0.0643	0.9	F
L2.1	L2.2	0.0633	0.0643	0.0546	F
L2.1	L2.3	0.0933	0.0643	0.004	T
L2.1	L2.4	0.0533	0.0643	0.1278	F
L2.1	L2.9	0.0167	0.0643	0.9	F
L2.10	L2.2	0.05	0.0643	0.1679	F
L2.10	L2.3	0.08	0.0643	0.0126	T
L2.10	L2.4	0.04	0.0643	0.3538	F
L2.10	L2.9	0.03	0.0643	0.6203	F
L2.2	L2.3	0.03	0.0643	0.6203	F
L2.2	L2.4	0.01	0.0643	0.9	F
L2.2	L2.9	0.08	0.0643	0.0126	T
L2.3	L2.4	0.04	0.0643	0.3538	F
L2.3	L2.9	0.11	0.0643	0.001	T
L2.4	L2.9	0.07	0.0643	0.0304	T
Reject F – False Reject T – True					

In the case of these groups, in addition to the fact that the absolute difference of the means exceeds the critical difference, both presented in Table 3, we can also observe that the value of p is less than 0.05, rejecting the null hypothesis which states that there is no significant difference between the two compared means.

The chart in Figure 7 shows the comparison between the absolute difference between groups averages and the critical difference.

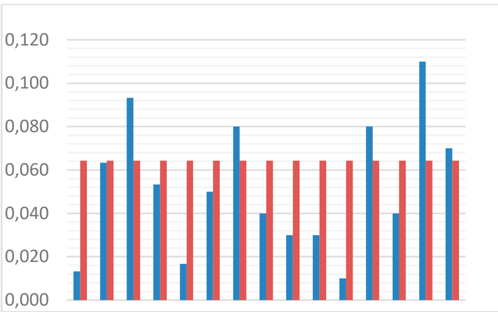

Figure 7. The difference between the absolute mean of the groups and the critical difference

Table 4. Moisture content results for the second batch

Crt.	T0			T1			T2	
Sample	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.	11.40	10.96	11.08	10.96	11.25	11.31	11.35	11.30
Average	11.18	10.99	11.10	10.99	11.26	11.22	11.37	11.28
SD	0.248	0.058	0.086	0.046	0.140	0.108	0.015	0.015

Assessing moisture content on the second batch

Table 4 shows the values obtained from the determinations made on the second batch produced. As in the case of the first batch, for T_0, three samples were randomly selected for analysis, being used in the homogeneity study. The analysis of the following samples, for T_1 and T_2, were also performed at an interval of 30 days.

The one-way ANOVA analysis was also used for this batch results to determine whether there were significant differences between the group means. The results obtained are presented in Table 5.

Table 5. One-way ANOVA result for the second batch

	DF	Sum of Square	Mean Square	F Statistic	P-value
Group	7	0.398667	0.056952	4.288852	0.007557
Residual	16	0.212467	0.013279	NaN	NaN
Total	23	0.611134	0.070231		

From these results, as in the case of the first batch, there can be observed significant differences between the 8 compared groups, highlighted by the graphical representation shown in Figure 8.

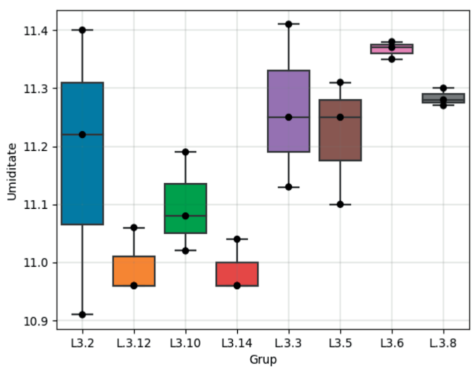

Figure 8. ANOVA chart for second batch

It is observed in Table 5 that F-value exceeds the critical F, as in the previous case and the value of p is well below the limit of 0.05, respectively 0.0075.

CONCLUSIONS

From the statistical interpretation of the results, it can be concluded that there is a need to carry out homogeneity and stability studies for each new batch produced even if the raw (starting) material is the same. The difference in results is determined by the moisture content of the raw material unprocessed and unpackaged for production.

The interpretation of the statistical data, obtained from the homogeneity studies and, respectively, the stability studies, were performed taking into account the test performance obtained within the MR laboratory from INCD IBA Bucharest.

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