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SELECTION OF THE TYPE OF WHEAT FLOUR IN THE DEVELOPMENT OF A REFERENCE MATERIAL FOR THE ANALYSIS OF THE WET GLUTEN CONTENT

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Abstract: Gluten content is one of the parameters that determines the quality of the bakery products, and it is analyzed with the help of the Glutomatic system. In order to develop a reference material for the determination of the wet gluten content, 5 different types of wheat flour were analyzed, namely: white wheat flour 480, white wheat flour 550, white wheat flour 650, black wheat flour 1350 and whole wheat flour. The results showed that white wheat flour 650 represents the optimum type of wheat flour for the development of a reference material for the wet gluten content determination, as this type of flour fulfilled the homogeneity conditions as well as the requirements imposed by SR EN ISO 21415-2:2016 for standard deviation of repeatability, critical difference and limit of repeatability between 2 measurements.
Keywords: wheat flour, wet gluten content, Glutomatic system, homogeneity

1. INTRODUCTION

Wheat flour is the product obtained by grinding the wheat after a preliminary cleaning. Gluten is the protein from wheat and is essential for bread making and influences the mixing, kneading and baking properties of the dough (*Rosell, 2011*). The gluten properties determine the dough characteristics and influence the quality of the final product. The amount and quality of gluten influence dough elasticity, gas retention, stretch/elasticity properties and will greatly influence the final baking quality. In addition, the ability to form a non-sticky dough, maintain the desired firmness of the dough and achieve consistent pasta cooking characteristics are all influenced by gluten properties (*Cauvain, 2021*).

The wet gluten is the viscoelastic substance that mainly contains 2 protein fractions (gliadin and glutenin) in a hydrated form. Glutenins give dough strength and elasticity, and gliadins give dough viscosity (*Rosell, 2011*). The gluten is isolated by washing the flour with a salt solution to remove starch and water-soluble fractions. Total wet gluten content is expressed as a percentage of the flour (*Tilley et al., 2012*). Thus, gluten is the residue left after the dough is washed and provides an indication of the quality of the flour.

The analysis of the wet gluten content in a flour sample can be carried out by a mechanical method or by a manual method according to *SR EN ISO 21415-2:2016* and *SR EN ISO 21415-1:2007*, respectively. In the case of the mechanical method, the Glutomatic system is the global standard for analysing the gluten quantity and quality (*Miś, 2000*).

The use of a reference material is necessary in the validation and evaluation of various measurements, as well as in the verification and detection of operational errors during analysis (*Budai and Fükõ, 2001*). A recent study presented the main requirements in the development of a quality system in the production of reference materials (*Serbancea et al., 2020*). Other studies focused on the influence of the food matrix and temperature in the development of a wheat flour reference material (*Marculescu et al., 2022a*; *Marculescu et al., 2022b*).

The aim of this study is to establish the type of wheat flour that can be used for the development of a reference material for the determination of the wet gluten content, using the mechanical method with help of the Glutomatic system. Thus, wheat flours with different ash content were analyzed from the point of view of the wet gluten content, namely: white wheat flour 480, white wheat flour 550, white wheat flour 650, black wheat flour 1350 and whole wheat flour.

2. MATERIALS AND METHODS

Wheat flour

Five different types of wheat flours in terms of ash content were evaluated for the wet gluten content, namely: white wheat flour 480, white wheat flour 550, white wheat flour 650, black wheat flour 1350 and whole wheat flour. The technical specifications for the analyzed wheat flours are presented in Table 1.

Figure 1 - The flasks with the flours for the analysis of the wet gluten content

The flour samples were without additives and packed in 5 kg paper bags. Flour samples were prepared according to the procedure described by

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Serbancea et al. (2021) with minor modifications. Briefly, the samples were first homogenized using an electromagnetic sieving equipment equipped with a 1.00 mm diameter sieve (model BN 300N, Cisa Cedaceria Industrial S.L., Barcelona). Afterwards, the samples were homogenized in a homogenizing mixer (model VS-8, Optic Ivymen System, Barcelona) and divided into 3 brown flasks of 100 g each (Retsch Sample Divider PT100, Germany), as shown in Figure 1. The flasks were stored at room temperature.

Table 1. Technical specifications for the wheat flours under investigation

	White wheat flour 480	White wheat flour 550	White wheat flour 650	Black wheat flour 1350	Whole wheat flour
Moisture, % max.	15.5	15.5	15.5	15.5	15.5
Acidity, ° max.	2.2	2.2	2.8	4	5
Wet gluten content, % min.	24	24	26	24	27
Protein content, % d.m. min.	11	-	10	11	11
Ash content, % d.m. max.	0.48	0.55	0.65	0.91 – 1.4	1.41 – 2.2
Falling Number, sec. min.	250	250	250	220	200
Particle size					
% max.	2[A]	2[A]	10[A]	8[A]	10[C]
% min.	65[B]	65[B]	55[B]	70[B]	70[A]

A: Residue on the sieve with 180 μm silk screen (no. 8); B: Pass through the sieve with 125 μm silk screen (no. 10)
C: Residue on the metal sieve with 0.5 mm

Determination of wet gluten content

The determination of the wet gluten content in the wheat flour samples was carried out according to SR EN ISO 21415-2:2016. Briefly, the method consists in preparing a paste from a sample of wheat flour with a sodium chloride solution in the equipment's chamber (Glutomatic equipment, Glutomatic 2200, Perten, Sweden) where the wet gluten is separated by washing this paste with the sodium chloride solution, followed by removal of excess washing solution by centrifugation and weighing of the residue.

Statistical analysis

For each sample, 3 replicate determinations were performed (3 flasks x 3 determinations). The results for the wheat flour analysis were presented as mean values together with standard deviation. Data were analyzed by one-way analysis of variance (ANOVA) with Tukey's test ($p < 0.05$).

3. RESULTS

The results obtained for the 3 flasks for each of the 5 types of flour samples are presented in Table 2. In order to study the variability of the measurements, the relative standard deviation (RSD (r)) was calculated using Equation (1):

$$\text{RSD (r)} = \frac{\text{standard deviation}}{\text{Average}} \cdot 100 \tag{1}$$

It is known that the more precise the results, the lower the RSD (Everitt and Skrondal, 2010). The results for the relative standard deviation showed small values: 1.8 – 2.6% (white wheat flour 480), 0.6 – 3.2% (white wheat flour 550), 0.5 – 1.3% (white wheat flour 650), 1.1 – 1.3% (black wheat flour 1350) and 1.2 – 3.9% (whole wheat flour), which means that the data on each vial is tightly clustered around the mean, so the data are not scattered. The lowest values for RSD were obtained for the white wheat flour 650.

Table 2. Wet gluten content in the wheat flour samples under investigation

Flour sample	Flask number	R1, %	R2, %	R3, %	Average*, %	SD	RSD (r), %
White wheat flour 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 wheat flour 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 wheat flour 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 wheat flour 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
Whole wheat 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

SD: standard deviation; RSD (r): relative standard deviation, %; R1: replication no. 1; R2: replication no. 2; R3: replication no. 3.
* The values that do not have the same letter are significantly different ($p < 0.05$; ANOVA with Tukey's test).

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In order to verify the homogeneity of the sample from the 3 flasks for each type of flour, the ANOVA test was applied, using the Tukey method (Table 2). In case of flour 480, flour 550, flour 650 and whole wheat flour, there were no significant differences in the gluten content ($p > 0.05$) between the 3 flasks, which means that the samples were homogeneous. On the other side, there were significant differences for gluten content between Flask 1 and Flask 3 ($p < 0.05$) for the black wheat flour 1350, showing the inhomogeneity of this flour.
The requirement established through the standard SR EN ISO 21415-2:2016 for the standard deviation of repeatability is 0.4. As seen in Table 2, only flour 650 and black flour 1350 for all 3 individual flasks fulfilled this requirement. According to the standard SR EN ISO 21415-2:2016, the critical difference ($d_{C,r}$) was calculated, which represents the deviation between two averaged values obtained from two test results in conditions of repeatability (Equation 2).

$$d_{C,r} = 2{,}8 \cdot S_r \cdot \sqrt{\frac{1}{2n_1} + \frac{1}{2n_2}} = 2{,}8 \cdot S_r \cdot \sqrt{\frac{1}{3}} \qquad (2)$$

where:
S_r is the standard deviation of repeatability;
n_1 and n_2 are the number of test results corresponding to each of the averaged values.
Thus, the average obtained on each of the 3 flasks was considered, and the results obtained using Equation (2) are presented in Table 3.

Table 3. Critical difference by comparing two sets of measurements

	F 1 vs. F 2	F 1 vs. F 3	F 2 vs. F 3
White wheat flour 480	0.3	0.5	0.8
White wheat flour 550	0.1	0.1	0.1
White wheat flour 650	0	0.2	0.2
Black wheat flour 1350	0.6	0.7	1.2
Whole wheat flour	0.1	0.9	0.7
Standard requirement*, %		0.8	

F: flask, *SR EN ISO 21415-2:2016

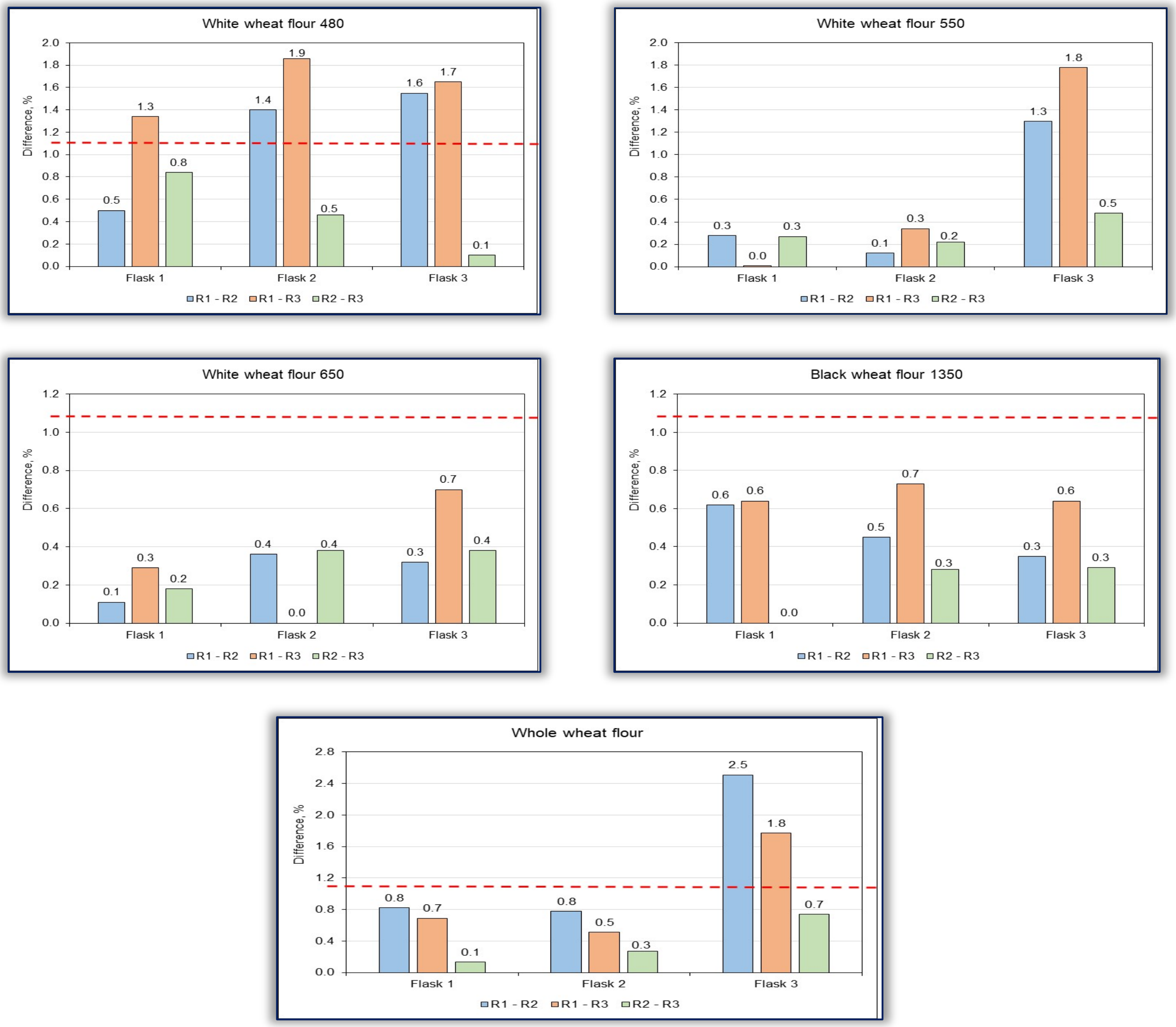

Figure 2 - Difference between replication determinations for gluten content for each of the 3 flasks (module values)
R1: replication no. 1; R2: replication no. 2; R3: replication no. 3.

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Figure 2 shows the difference between replications on each individual flask. The requirement imposed by the standard SR EN ISO 21415-2:2016 for the limit of repeatability between two independent measurements obtained in the same laboratory, by the same analyst, is 1.1. As it can be seen, white wheat flour 650 and black wheat flour 1350, met this criterion for all the three flasks.

4. CONCLUSIONS

Gluten is the protein in wheat which is responsible for the baking properties of the wheat flour. In order to develop a reference material for the analysis of the wet gluten content, 5 different types of wheat flour were considered: white wheat flour 480, white wheat flour 550, white wheat flour 650, black wheat flour 1350 and whole wheat flour.

White wheat flour 650 presented the lowest values for the standard deviation (between 0.5 - 1.3%) for the 3 flasks compared to the other types of flour analyzed.

Calculation of the critical difference by comparing two sets of measurements showed that white wheat flour 480, white wheat flour 550 and white wheat flour 650 met this condition for each of all the three flasks.

ANOVA analysis with Tukey's test showed that there were no significant differences between the 3 flasks ($p > 0.05$) in case of white wheat flour 480, white wheat flour 550, white wheat flour 650 and whole wheat diet flour, which means that the flasks were homogeneous.

The requirements imposed by SR EN ISO 21415-2:2016 were verified, namely: the standard deviation of the repeatability, the repeatability between two independent measurements and the critical difference between two measurements in the determination of the wet gluten content, which are met only by white wheat flour 650. Also, white wheat flour 650 met the homogeneity conditions. Therefore, among the analyzed flours, white wheat flour 650 represents the optimal type of wheat flour for the development of a reference material for the determination of the wet gluten content.

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