

THE INFLUENCE OF THE SPROUTED WHEAT KERNELS (SWK) ON THE AMILOLYTIC ACTIVITY OF THREE WHEAT VARIETIES, CULTIVATED IN ROMANIA

Ciprian-Nicolae Popa ¹, Radiana-Maria Tamba-Berehoiu ^{2*},
Luminita Valerica Visan ², Vasilica Simion ²

¹Farinsan SA, Gradistea village, Giurgiu district, Romania

²University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59, Marasti Blvd,
District 1, Bucharest, Romania,

Abstract

The Falling number FN (s) and the Sprouted Wheat Kernel SWK % (ISO 3093, SR ISO7979:2001) were analyzed for 3 varieties of wheat, Dropia, Flamura 85 and Fundulea 4, mainly cultivated in the southern region of Romania. The descriptive statistics highlighted the extremely high variability coefficients of SWK, namely: 182.93 % at Dropia, 99.35 % at Flamura 85 and 152.60 % at Fundulea 4 variety. The t test did not reveal significant differences between the SWK means and between the FN means in the three varieties, with the exception of FN means between Dropia and Fundulea 4 ($t=2.410^*$; $F \text{ ratio}=1.246^*$; $p<0.05$). The Shapiro-Wilk normality test of FN and SWK distributions, highlighted that FN distribution at Dropia, SWK at Flamura 85 and SWK at Fundulea 4, were not normal ($w=0.871^{***}$, 0.784^{***} , 0.638^{***} ; $p<0.01$). Spearman correlations between FN and SWK were negative significant in all wheat varieties (-0.521^{**} at Dropia, -0.373^* at Flamura 85 and -0.688^{**} at Fundulea 4). The best regressions models, describing the relationship between FN and SWK in the Dropia and Fundulea 4 varieties, were exponential respectively logarithmic ($r^2 = 0.75$; $r^2 = 0.59$). For the Flamura 85 variety, no suitable regression model was identified ($r^2 = 0.15$). The maximum SWK level at which minimum wheat bakery quality was achieved (FN = min. 180 s) was 2.95 % for Dropia and 1.86 % for Fundulea 4.

Keywords: Dropia variety, falling number, Flamura 85 variety, Fundulea 4 variety, sprouted wheat kernels.

1. INTRODUCTION

Wheat bakery qualities are significantly affected by the environmental conditions in which crop develops (Johansson, 2002). For example, Romanian wheat harvests are occasionally affected by the kernels sprouting in the wheat ear. This is due to the fact that autumn wheat harvesting takes place in Romania between June and July, a period characterized by meteorological statistics, as the most rainy of the year (Ștefănescu and Stefan, 2011; Cheval et al., 2011). The kernels sprouting is accompanied by a series of physiological changes that affect the wheat bakery quality. For example, triggering the amylolytic activity necessary for embryo growth, due to starch degradation in the endosperm, involves the synthesis and activation of enzymes (amylases, proteases) that resist to the milling process and remain in the flour. These enzymes act in the sense of starch and gluten degradation in the dough phase, modifying negatively its technological characteristics (Mares et al.,

2008; Wrigley, 2008). The products obtained from these doughs are characterized by a low volume, an intense coloration of the crust, a dense, sticky and gluey core (King, 1989; Derera, 2018).

The technological challenges, induced by excessive amylase activity in wheat, can not generally be solved by using additives, these kind of cereals being unsuitable for using in the bakery industry (Popa, 2007). Most of the corrections that have been proposed to solve this problem had the objective to modify the doughs pH, by using either acidifiers (organic acids or salts thereof) or alkalinizers (sodium hydroxide salts) (Tamba-Berehoiu et al., 2011). However, both dough pH correctors imply deviations from the pH values at which dough fermentation processes are usually conducted in industry, with repercussions on the quality of finished products. The parameter characterizing the amylase activity of the wheat is the Falling number (FN). This parameter may take values between 62 and over 500 seconds and is inversely proportional to amylazic activity (Perten, 1964; MacArthur et al., 1981; Finney, 1985; Kweon, 2010). Modern wheat quality standards set a minimum value for this parameter between 180 and 200 seconds, below which wheat is considered unsuitable for bakery (Alaru et al., 2003; Tilley et al., 2012). Generally, in years with a normal climate regime, Romanian wheat crops are characterized by Falling number values of more than 350 seconds (Popa et al., 2008). Taking into account the above aspects, the main solution to this problem can be provided by the cultivation of wheat genotypes characterized by high sprouting resistance (Flintham et al., 2002; Brown et al., 2018).

The purpose of the paper was to determine to what extent the percents of Sprouted Wheat Kernels (SWK) influence the amilolytic activity of the three varieties of wheat (expressed by the Falling number parameter).

2. MATERIALS AND METHODS

We analyzed comparatively the influence of the sprouted wheat kernels (SWK) on the amilolytic activity of three wheat varieties, cultivated mainly in southern Romania, namely: Dropia, Flamura 85 and Fundulea 4. Autumn Dropia variety was obtained at Fundulea INCD through repeated individual selection. The plant bush is half- upright in the twining phase. The kernels are of medium size, elongated shape, red color and the mass of 1000 kernels is 41-46 g (hectoliter weight 76-80 kg/hl) (INCDA Fundulea, 2018). Wheat variety Flamura 85 is a powdery mildew disease resistant variety obtained from the mixture of five phenotypically similar lines. The mass of 1000 kernels is 44-46 g (hectoliter of 76-80 kg/hl). The kernels have the shape and color of the Dropia variety (INCDA Fundulea, 2018). Fundulea variety 4, has phenotypic characteristics similar to Dropia and Flamura 85, but gluten is softer and the sedimentation index is lower. The average height of plants in the three varieties is similar, being 85-95 cm (Saulescu et al., 2007; Mustătea et al., 2009; Lugojan et Ciulca, 2011).

In this regard there have been analyzed 36 samples of Dropia and Flamura 85 wheat varieties and 19 samples of Fundulea 4 variety, in terms of Falling number FN (s) and Sprouted Wheat Kernels SWK% (ISO 3093:2009 - Wheat, rye and their flours, durum wheat and durum wheat semolina). FN analysis, according to Hagberg-Perten, was made as defined in SR ISO7970: 2011 – Specifications. The proportion of SWK was determined from 100 grams wheat sample (according to SR ISO 24333: 2009 - Cereals and cereal products – Sampling).

The wheat samples came from the Romanian crops of the years 2012-2017. The results obtained were interpreted using the StatSoft Statistica v. 7, professional program.

3. RESULTS AND DISCUSSIONS

The descriptive statistics of analyzed parameters, FN and SWK, are presented in Table 1.

Table 1. Mean values and variability estimates of FN (s) and SWK (%)

Wheat varieties	Parameter	Mean(X)	Stand. dev.(s _x)	s (variance)	Variab. coeff.(CV%)
Dropia (n=36)	FN	319.416	± 82.081	6737.290	25.690
	SWK	0.744	± 1.361	1.852	182.930
Flamura 85 (n=36)	FN	304.083	± 68.183	4648.935	22.420
	SWK	0.616	± 0.612	0.374	99.350
Fundulea 4 (n=19)	FN	261.000	± 91.646	8398.989	35.110
	SWK	1.000	± 1.526	2.331	152.600

The descriptive statistics highlighted an extremely high variability coefficients of SWK, namely: 182.93% at Dropia, 99.35% at Flamura 85 and 152.60% at Fundulea 4 variety. Amilolytic activity of wheat, expressed by Falling number depends on many factors (vegetation period, rawness, enzyme activators, temperature etc.), fact which explains the increased variability of this parameter. Extremely high variability suggested that the parameters are not normally distributed. Generally, the extreme variability of the parameters associated with enzymatic activity reflects their sensitivity to environmental factors.

In Table 2 is highlighted the normality Shapiro-Wilk test for FN and SWK.

Table 2. The Shapiro-Wilk normality test for FN (s) and SWK (%)

Parameter	Median	Mean-Median difference	W	p
FN				
Dropia	346.00	319.416 - 346.000 = - 26.584	0.871***	0.000
Flamura 85	300.50	304.083 - 300.500 = 3.583	0.973	0.522
Fundulea 4	288.00	261.000 - 288.000 = - 27.000	0.949	0.384
SWK				
Dropia	0.300	0.744 - 0.300 = 0.444	0.470	0.000
Flamura 85	0.350	0.616 - 0.350 = 0.266	0.784***	0.000
Fundulea 4	0.300	1.000 - 0.300 = 0.700	0.638***	0.000

*significant at $p < 0.05$; **very significant $p < 0.01$; ***extremely significant $p < 0.001$

The Shapiro-Wilk normality test of FN and SWK distributions, highlighted that FN distribution at Dropia, SWK at Flamura85 and SWK at Fundulea4, were not normal. The deviations from normality being extremely significant ($w=0.871***$, $0.784***$, $0.638***$, $p < 0.001$).

Significant differences between wheat varieties, concerning FN (s) and SWK (%), were tested using the t (Student) test (Table 3).

Table 3. The significance of means and standard deviations differences of FN (s) and SWK (%) between wheat varieties

Group 1 vs. Group 2	Mean	Mean	t-value	df	p	Std.Dev.	Std.Dev.	F-ratio	p
FN Dropia vs. Fundulea	319.416	261.000	2.410*	53	0.019	82.081	91.646	1.246*	0.560

*significant at $p < 0.05$; **very significant $p < 0.01$; ***extremely significant $p < 0.001$

The t test did not reveal significant differences between the SWK means and between the FN means in the three varieties, with the exception of FN means between Dropia and Fundulea4 ($t=2.410^*$; $F \text{ ratio}=1.246^*$; $p<0.05$).

Within each wheat variety, specific metabolic correlations were established between the FN and SWK parameters. Table 4 shows the Spearman significant correlations.

Table 4. Spearman correlations coefficients between FN and SWK for every wheat variety

Wheat varieties	FN -SWK	
	r	p
Dropia	-0.521**	0.0011
Flamura 85	-0.373*	0.0250
Fundulea 4	-0.688**	0.0011

*significant at $p<0.05$; **very significant $p<0.01$; ***extremely significant $p<0.001$

Spearman correlations between FN and SWK were negative significant in all wheat varieties (-0.521** at Dropia, -0.373* at Flamura 85 and -0.688** at Fundulea 4).

The best regressions models, describing the relationship between FN and SWK in the Dropia and Fundulea 4 varieties, were exponential and logarithmic ($r^2=0.75$; $r^2=0.59$). For the Flamura 85 variety, no suitable regression model was identified ($r^2=0.15$) (figure 1).

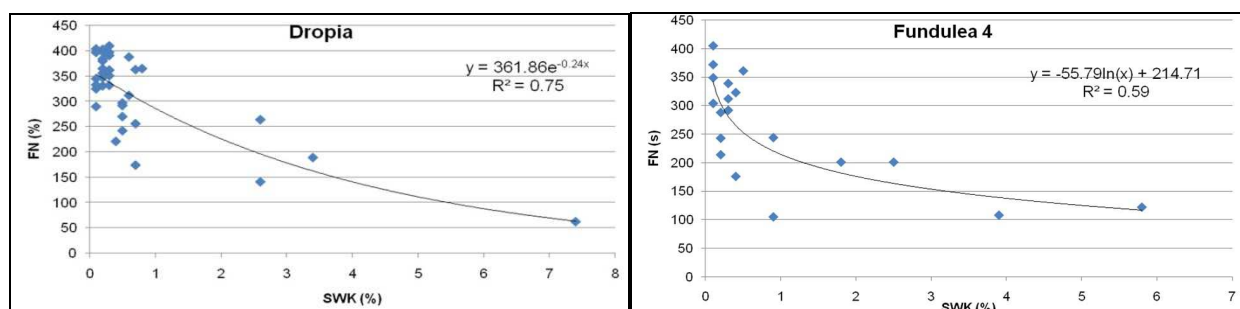


Figure 1. The regressions models, describing the relationship between FN and SWK in the Dropia and Fundulea 4 varieties

The results emphasize the importance of choosing a genotype appropriate to the pedoclimatic conditions, characteristic of wheat crop areas, when it comes to obtaining products that can be easily utilized by the bakery industry. As we have seen before, the wheat harvesting period in the southern area of Romania, corresponds to that of the year, characterized by the recording of the highest pluviometric values. Basically, the wheat harvesting period corresponds to the ideal conditions for triggering germination of kernels in wheat ear and implicitly to the appearance of dough quality problems caused by this phenomenon, namely: high amylase activity, reduced gas retention capacity, and reduced mechanical stress resistance.

It should be noted that wheat characterized by a very high amylase activity can not be used as such, but only in mixtures, whose percentages can not be calculated by using the weighted means or simple arithmetic means of the FN parameter. Thus, the use of 5% wheat with a Falling number of 62 can easily compromise a mixture of 95% wheat, characterized by a Falling number of over 300 s. This thing is happening because enzymatic activity (determined by a multitude of conditions such as the amount of substrate and its nature, pH, temperature etc.) is not proportional to the amount of enzyme, and the relationship between the enzymatic activity and the viscosity of the starch gel, used to determine the Falling number, is logarithmic rather than linear. Moreover, the flours coming

from these wheat types are difficult or almost impossible to correct, using chemical additives approved by the European legislation in force, in the technological processes of bread obtaining (Tamba-Berehoiu et al., 2011).

Normally, the destiny of such wheat crops involves their use in animal feed schemes. The use in the technological processes of the milling industry, determines besides the bakery problems concerning the flours, wheat flour yield losses (the amount of flour obtained from 100 kg of wheat) and appearance of some altered sensorial characteristics (Costin, 1983). In addition, wheat characterized by a very high amylase activity is frequently associated with the increase in the incidence of mycotoxin-producing fungi (*Fusarium* spp.).

We believe that our work may be a starting point for an adequate analysis of available genotypes, characterized by resistance to climatic conditions that triggers amylase activity in wheat. The selection and promotion of resistant genotypes can be of a real benefit to the agriculture, milling and bakery industry in Romania, by avoiding additional management-related costs incurred for their exploitation.

4. CONCLUSIONS

Our results suggest that the three analyzed wheat varieties respond differently to changes in amylase activity relative to the proportion of Sprouted Wheat Kernels. Thus, the variety best preserving the bakery properties, correlated with the amylase activity, was the Dropia variety, and the most sensitive variety to the Sprouted Wheat Kernels percent was Fundulea 4. No significant regression model was found for Flamura 85 wheat variety, between the Falling number and the Sprouted Wheat Kernels percent. The maximum SWK level at which minimum wheat bakery quality was achieved (FN=min 180 s) was 2.95% for Dropia and 1.86% for Fundulea 4.

6. REFERENCES

- Alaru, M., Laur, Ü., Jaama, E. (2003). Influence of nitrogen and weather conditions on the grain quality of winter triticale. *Agronomy Research*, 1(1), 3-10.
- Brown, L. K., Wiersma, A. T., Olson, E. L. (2018). Preharvest sprouting and α -amylase activity in soft winter wheat. *Journal of Cereal Science*, 79, 311-318.
- Cheval, S., Baciú, M., Dumitrescu, A., Breza, T., Legates, D. R., Chendeş, V. (2011). Climatologic adjustments to monthly precipitation in Romania. *International Journal of Climatology*, 31(5), 704-714.
- Costin, I., (1983). Tehnologii de prelucrare a cerealelor în industria morăritului [Grain processing technologies in the milling industry]. Ed. Tehnică, Bucureşti.
- Derera, N. F. (2018). *Preharvest Field sprouting in Cereals*: 0. CRC Press.
- Finney, P. L. (1985). Between Falling Numbers and Alpha-Amylase Activity. *Cereal Chem*, 62(4), 258-262.
- Johansson, E. (2002). Effect of two wheat genotypes and Swedish environment on falling number, amylase activities, and protein concentration and composition. *Euphytica*, 126 (1), 143-149
- King, R. W. (1989). Physiology of sprouting resistance. *Preharvest Field Sprouting in Cereals*, 27-60.
- Kweon, M. (2010). Falling number in wheat -How is it calculated and what does it mean to producers? Meera Kweon. USDA. ARS. Soft Wheat Quality Lab. Wooster. OH., USA., [http://www.ars.usda.gov/SP2UserFiles/Place/36070500/InfoDianehasuploaded/2010 Research Review AnnualReport/MKweon-FN-012810.pdf](http://www.ars.usda.gov/SP2UserFiles/Place/36070500/InfoDianehasuploaded/2010%20Research%20Review%20AnnualReport/MKweon-FN-012810.pdf)
- Lugojan, C., Ciulca, S. (2011). Evaluation of relative water content in winter wheat. *Journal of Horticulture, Forestry and Biotechnology*, 15(2), 173-7.
- MacArthur, L. A., D'Appolonia, B. L., Banasik, O. J. (1981). The falling number test: what is it and how does it work? [To detect sprout damage in wheat, hard red spring and durum wheat]. *North Dakota Farm Research*.
- Mares, S., Daryl, J., Mrva, K. (2008). Late-maturity α -amylase: Low falling number in wheat in the absence of preharvest sprouting. *Journal of Cereal Science*, 47 (1), 6-17.
- Mustatea, P., Saulescu, N. N., Ittu, G., Paunescu, G., Voinea, L., Stere, I., Nastase, D. (2009). Grain yield and yield stability of winter wheat cultivars in contrasting weather conditions. *Romanian Agricultural Research*, 26, 1-8.

- Perten, H. (1964). Application of the falling number method for evaluating alpha-amylase activity. *Cereal chem*, 41(3), 127-140.
- Popa, N.C., (2007). Influența unor amelioratori de origine vegetală și microbiană asupra parametrilor de calitate ale făinurilor din grâu [Influence of plant and microbial breeders on the quality parameters of wheat flour], Doctoral thesis, Facultatea de Horticultură, Universitatea de Științe Agronomice și Medicină Veterinară, București.
- Popa, N.C., Tamba-Berehoiu, R., Popescu, S. (2008). Main quality parameters' evolution of the wheat cultivated in Romania during the last years. *Roumanian Biotechnological Letters*. 13(1), 3557-3566.
- Săulescu, N. N., Ittu Mariana, Mustătea P. (2007). Cinci decenii de ameliorare a grâului la Fundulea [Five decades of wheat improvement in Fundulea], *Analele INCDA Fundulea*. LXXV volum jubiliar, 65-66.
- Stefanescu, V., Stefan, S. (2011). Synoptic context of floods and major flash floods in Romania during 1948–1995. *Romanian Reports in Physics*, 63(4), 1083-1098.
- Tamba-Berehoiu, R., Popescu, S., Popa, C. (2011). The Effect Of Preparates Based On Sodium Carbonate And Citric Acid On The Parameter Falling Number In Hyperdiastazic Flours. *Scientific Bulletin*, 3, 92.
- Tilley, M., Chen, Y. R., Miller, R. A. (2012). *Wheat breeding and quality evaluation in the US*. In *Breadmaking* (Second Edition, pp., 216-236).
- Wrigley, C. (2006). Late-maturity α -amylase – apparent sprout damage without sprouting. *Cereal Foods World*, 51 (3), 124–125.
- ***Dropia, INCDA Fundulea 2018, <http://www.incda-fundulea.ro/fise/dropia.pdf>, downloaded at February 23 2018
- ***Flamura 85, INCDA Fundulea, 2018, <http://www.incda-fundulea.ro/fise/f85.pdf>, downloaded at February 23 2018