Influence Of Fusariosis In The Biochemical And Rheological Properties Of Different Wheat Cultivars

Dorina Beli[*], Artiona Laze, Merita Stafasani
Department of Chemistry, Agriculture University of Tirana, Tirana, Albania
[*]dorinabeli9@gmail.com

Gaetano.Cardone,
Department of Food, Environmental and nutritional Sciences (DeFEnS), Università degli Studi di Milano, 2, Via giovanni Celoria, 20133 Milan, Italy

Skender Varaku
Department of Plant Protection, Agriculture University of Tirana, Tirana, Albania.

Evelina Ceca
College of Science and Mathematics, University of Massachusetts, Boston, USA.

Abstract—Fusarium **spp. infection of cereal grain is a common problem, which can result in a decline of grain quality. The objective in this study was to understand the correlation between different degrees of infestation and the quantitative-qualitative changes in flour. The effects of different grades of infection of *Fusarium* were evaluated on: thousand kernel weight, wet gluten (WG), protein content, Zeleny sedimentation (ZS) and Falling Number (FN). Some of the most important rheological tests used in the industry were conducted on wheat flour of all varieties: Mixolab and GlutoPeak Test. The disease index average was 14.07 % varying from 4.07 % to 34.3% .The crude protein content was not significantly affected by the *Fusarium* spp. infection; on the contrary, FN, ZS and WG showed distinctively decreased values in all the infected cultivars.**

Keywords— *Fusarium Head Blight,wheat, disease index, proteins, gluten.*

I. Introduction

Wheat is a cereal of critical importance in the global cereal production. The edible wheat grain has to demonstrate a high technological value warranting stability of technological processes and good quality of bakery and other products. One of the factors that can adversely affect quality parameters of grain is the presence of grain diseases caused by fungal infestation [1]. In Albania the total area planted with grain is 69.600 hectares, almost a quarter of the area planted before 1990. The total wheat production for 2015 was 275.000 tones [2]. FHB is one of the most economically important diseases of wheat and causes yield losses, grain quality reduction and contamination on grain with mycotoxins [3]. Infection from the fungus during anthesis of wheat can afflict starch qualities and it results in a higher degree of damaged starch gluten proteins and albumin [7]. Also it has a high susceptibility against α amylase activity.

This could be a negative factor during the bread making process because the infection *Fusarium spp* produces a large amount of amylase and it can lead

to production of sticky and weak dough [5]. The new apparatus Mixolab [32], enables evaluation of physical dough properties such as dough stability or weakening and starch characteristics in one measurement by intense mixing and controlled heating of the kneader to 90°C and ensuing cooling to 50°C [6]. Mixolab curve is separated into five stages, characterized by five points (C1–C5) and other parameters resulting from the differences between the individual points (Fig.1). Characteristics evaluated from the measured Mixolab curve are:

C1 used to determine water absorption

C2 represents the weakening of the protein based on the mechanical work and the increasing temperature

C3 represents the rate of starch gelatinization

C4 represents the stability of the hot-formed gel

C5 represents starch retrogradation during the cooling period C1–C2 indicates the protein network strength under increasing heating

C1–C2 indicates the protein network strength under increasing heating

C3–C4 shows diastatic activity and relates with falling number

C4–C5 correlates with the anti-staling effects, represents the shelf life of the end products; dough stability indicates the stability of the dough before weakening.

The two first stages of the curve correspond to the rheological characteristics of stability, elasticity, and water absorption, related to proteins. The consistency of dough, which decreases with excessive mixing, is an indication of protein weakening. The faster and greater the decrease of the dough consistency indicates the lower the protein quality [6]. *F. graminearum* infection can cause significant changes in carbohydrate, lipids and proteins composition which can be observed in different values of Mixolab parameters. Additional changes also seriously affected the quality of storage proteins and dough properties [8]. During the colonization of the kernel from the fungus an increase of protease and amylase activity was observed. According to Boyacioglu and Hettiarachy, Fusarium is an aggressive invader which can attack starch granules and gluten proteins because of production of enzymes form the fungus [9]. Gluto Peak is a rapid way to measures gluten quality in cereal flour products. It only requires a small amount of sample and it takes only 10 minutes to give an estimation of gluten quality. The GlutoPeak tester is a high shear based technique that provides two attributes of gluten quality; torque that is an indication of strength of gluten and time to peak that is an indication of kinetics of gluten aggregation [14]. Description of the evaluation Retention time (RT) (Peak maximum time), time required for gluten to aggregate and exhibit¬ maximum torque on the paddle before breaking down Peak value (PV) Maximum torque in BU (Brabender® Units)¬ High peak: High content of strong gluten. Low or no peak: Low content of gluten (Brabender GlutePeak manual) [35].The study was focused on the detection of qualitative changes in wheat grain with a different intensity of *Fusarium spp*. contamination using standard methods for technological quality determination and rheological evaluation by the system Mixolab and GlutoPeak. Also the aim of this study is to correlate the effect of different infection grades with agro-biological parameters of wheat (ash, moisture, thousand kernel weight, protein, K-SDS sedimentation and falling number) which are important for the good quality of bread. The experiment was set on naturally contaminated wheat. Material and Methods

II. MATERIALS AND METHODS

A. Study area and plant sampling

The effect of different infection grades of *Fusarium spp* was evaluated for nine winter wheat cultivars samples planted during the years 2015-2016 on experimental fields of the State of Seed and Saplings (ATTC) in Lushnje (40°57′06″N, 19°41′08″E) .The plant material was naturally contaminated by *Fusarium* infection during growth in the field. Field trials were carried out by methods of randomized blocks in four replications; the size of each plot was 10 square meters. For visual estimation for each plot, 10 heads were taken, at five places of, its diagonals, resulting in a total of 50 heads per plot. Agro-technical practices have been the same based on the protocol type established previously for the distance of planting, seed rate, doses of fertilizers, hoeing, etc.

B. Plant Materials

Nine soft wheat cultivars; Dajti, Lucia, Katerina, Mateo, Apache, Mia, Azul, and Krajlica and Europa have been selected for investigation and cultivated in the same location at experimental fields of the State of Seed and Saplings (ATTC) Lushnja.

C. Desease assessment

Times Assessments: Assessment of Fusarium spp was made two times each Zadoks growth stage GS 60 and GS 75 [10]. Two measures were obtained per spike disease assessment: incidence (presence or absence of disease anywhere on the plant) and severity (percentage of each spike with disease symptoms).

Visual Estimation: Visual estimation of disease severity from natural infection Fusarim Head Blight (caused by *Fusarium spp*) was used. Assessment of *Fusarium* infections has been based on standard area diagrams (SADs) the percent of covered leaves surface occupied by the disease. (Images for SADs created using Severity Pro software10. (*See Fig. 1*). Severity and diffusion of infection were obtained by resorting to the McKinney index [11, 12] and modified from Cooke, 2006 [13]. The McKinney index ([Imc (%)] was obtained by using the following formula:$=\Sigma\{(n_i \cdot x_i)/N \cdot X\} \cdot 100$, where: ni = infection class frequencies; xi = number of plants of each class; N = total of observed plants; X = highest value of the evaluation scale.

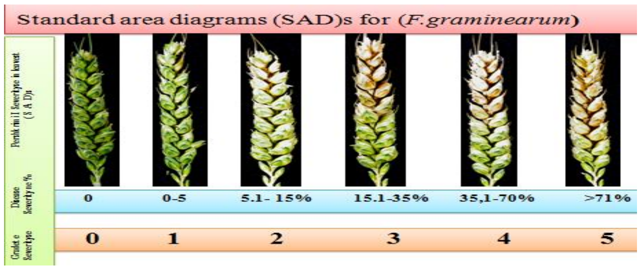

Fig.1.*Standard area diagrams used to estimate FH (Fusarium spp).*

D. Chemical and Technological Analyses

Moisture content was analyzed using the AOAC method [33]. Crude protein content was analyzed (CP) according to the Kjelhdahl method (N x 5.7). The Wet gluten content (WG) in grain dry matter was determined using Glutomatic Perten, and it was analyzed according to the AACC method 38-12.02 [34]. The sedimentation value (K-SDS) was determined according to Zeleny, 1947 [12]. HFN was determined in accordance with PN-ISO-3093, which follows the procedure as described in the standard procedures of the International Association of Cereal Science and Technology (ICC) No. 107. Thousand kernel of weight were determined by the methods described by Dexter and Tipples [28].

E. Mixolab determination

Rheological characteristics were determined using the apparatus Mixolab® Chopin, for white flour [29]. Point C1 was not included in the final evaluation because this side point is sufficiently represented by the difference between points C1 and C2 (C12).

F. Statistical Date Analysis:

All statistical analyses were performed using Minitab 17 and Excel 2007.

III. RESULTS AND DISCUSSION

The chemical – technological parameters of nine cultivars of wheat grain are presented in Table 1. In the nine analyzed cultivars the scale of infection ranges from high to moderate.

A. Thousand kernel of wheat (TKW)

Thousand kernel of wheat ranged from 37 g (Dajti) to 48.98 g (Lucia). Dajti is an Albanian autochthones cultivar and it is well established in Albania in climatic condition. A thousand kernel weight for Dajti ranges from 43-45 g. So we can presume that it was a reduction of 1000 kernel weight. Also this cultivar has the highest infection degree. A higher TKW and higher test weight means a greater proportion of endosperm in the seed and thus a better yield of flour [12]. This result is in accordance with other studies [27], which suggest a dramatic decrease of TKW due to *Fusarium* head blight infection. This reduction may be as result of consumption of starch and storage proteins from the fungus. Infected spikelet's yield seeds with characteristic visual symptoms they have a pink, chalky white, or pale gray color and a" tombstone" appearance. [19,20]. In highly susceptible cultivars, 50 to 80% of developing spikelet's may be killed or damaged prior to grain fill [16,17].

B. Ash

An increase of ash content of flour was recorded in the most infected cultivars (Dajti 2.16%, Apache 2.1 and Katerina 2.18%). Ash is mainly composed of minerals of the seed coat (bran and aleurone), the proportion of the ash in flour is an indicator of its purity. An increase in ash content means an alteration on the kernel seed coat volume ratio. It is possible to associate this estimation with the presence of shriveled *Fusarium* damaged kernels [15]. We have found a medium correlation coefficient (r=0.7) between ash and disease index (Imc) table II.

TABLE I. *THE CHEMICAL- AGRO-BIOLOGICAL PARAMETERS OF NINE CULTIVARS*

Cultivars	Ash (%)	Moisture (%)	TKW (g)	Imc (%)	K-SDS (ml)	Proteins (%)	WG (%)
Dajti	2.16	11.44	37	34.3	29.5	14.07	16.1
Lucia	1.85	11.4	48.98	5	40.4	12.6	22
Mia	1.6	11.32	45.52	15.7	33	13.07	19.5
Katerina	2.18	11.85	42.5	11.7	30.2	14.96	20
Europa	1.65	11.8	43.12	6	39.8	11.1	22
Asul	1.54	11.6	42.76	4.07	40.2	10.6	24
Krajlica	2	11.34	37.2	16.8	29.7	13.9	10
Mateo	1.44	11.48	41.44	4.07	37.9	13.8	24.9
Apache	2.1	10.8	40.28	29	28.4	14.36	20.1

C. Crude proteins

The amount of crude proteins increased slightly in the most affected cultivars. The correlation coefficient between crude proteins and disease index was 0.54. FHB alters the quality of proteins [16]. In recent studies the infection grade did not affect in particular the crude protein content [17]. According reference [18] the protein content increases significantly during a severe infection. Also they suggest that *Fusarium* destroys starch granules because of the production of amylase and protease from the fungus during infection and it can result in increase of protein and ash content in percentage but also we can detect a deterioration of protein quality. In this study, it was observed that there was an increase in the value of proteins content which ranged from 10.60% (Asul) to 14.96 % (Apache).

D. Sedimentation Value

K-SDS measures the swelling potential of the kernel proteins in lactic acid. Cultivars with highest infection degree manifested a lower sedimentation value. Grain with values of the coefficient of sedimentation between 0-25 ml are considered weak, between 25-40 ml are in a mean range and good grains are considered those with this value above 40 ml. In our cultivars it ranges from 28.4 ml (Apache) with a severe infection rate up to 40.4 ml (Lucia) with the minimum infection rate. We have found a strong negative correlation coefficient (r=-0.831, p=0.006) between K-SDS and disease index (Imc). According reference [19] a general reduction of K-SDS after FHB infection was observed. Although the total amounts of proteins remains stable, FHB alters the quality of proteins [16].

E. Wet gluten

The wet gluten test measures the amount of gluten protein in flour. According references [20, 31] the content of wet gluten in undamaged Albanian cultivars ranges from 23.43% to 33.20%, with an average value of 28.94% [20]. Other studies found wet gluten content reduced in artificially *Fusarium* infected samples. [9,19]. In the analyzed samples, specifically Dajti (Imc =34%), and Apache (Imc =29 %), we have found the lowest value for wet gluten content. According reference [1], there was an increase of wet gluten in damaged grain compared to healthy kernels with significant differences. Those different results can be attributed to the fact that we don't know when the colonization from the fungus happened. Glutenins are synthesized more rapidly than gliadins during later stages of kernel maturation [21].

F. Falling number

The level of enzyme activity measured by the Falling Number Test affects product quality. A high falling number (above 300 seconds) indicates minimal enzyme activity and sound quality wheat or flour and a low falling number (below 250 seconds) indicates substantial enzyme activity and sprout-damaged wheat or flour. It is, however, well recognized that the falling number depends upon the prevailing climatic conditions. Meyer and Weipert [30] found the falling number decreased only in one out of 3 years. In conclusion, in a breeding context, the variation of the falling number after FHB infection cannot be used as a selection criterion. [22]. Correlation between falling number and disease index has not been found, but the value of this trait was lower for the most damaged cultivar Dajti 225(s); and Apache 254(s) with the highest infection degree. One can presume that in the cultivars Dajti and Apache, there is a higher level of enzyme activity and the starch is damaged as a result of the production of fungal α amylase.

TABLE II. *CORRELATION COEFFICIENT BETWEEN CHEMICAL-AGRO-BIOLOGICAL PARAMETERS AND MIXOLAB CURVES*

Correlation	Imc	FN	C1	C2	C3	C4	C5	Ash	TKW	K-SDS	Prot	WG
FN	-0.458 0.215	1										
C1	0.330 0.385	-0.058 0.882	1									
C2	-0.770 0.015	0.636 0.066	-0.365 0.334	1								
C3	-0.392 0.296	0.449 0.225	0.337 0.376	0.535 0.138	1							
C4	-0.748 0.020	0.531 0.141	-0.043 0.912	0.783 0.012	0.877 0.002	1						
C5	-0.591 0.094	0.590 0.094	-0.727 0.026	0.797 0.010	0.010 0.980	0.360 0.341	1					
Ash	0.701 0.035	-0.764 0.016	0.031 0.912	-0.781 0.013	-0.693 0.038	-0.802 0.009	-0.462 0.210	1				
TKW	-0.626 0.071	0.092 0.813	-0.389 0.301	0.583 0.099	0.442 0.233	0.706 0.034	0.406 0.278	-0.420 0.261	1			
K-SDS	-0.831 0.006	0.493 0.178	-0.149 0.702	0.854 0.003	0.781 0.013	0.961 0.000	0.463 0.209	-0.769 0.015	0.646 0.060	1		
Prot	0.549 0.126	-0.231 0.550	0.106 0.786	-0.697 0.037	0.659 0.054	-0.743 0.022	-0.243 0.529	0.664 0.051	-0.404 0.281	-0.812 0.008	1	
WG	-0.562 0.115	0.399 0.287	-0.211 0.586	0.743 0.022	0.595 0.091	0.675 0.045	0.562 0115	-0.602 0.086	0.601 0.087	0.694 0.038	-0.432 0.246	1

G. Mixolab determination:

Information concerning mechanical and thermal protein network weakening, starch gelatinization and starch gelling can be found by the Mixolab curves recordings. From Table 2 it can be observed that the lowest of C2 (C2 represents the weakening of the protein based on the mechanical work and the increasing temperature) parameters were obtained by the cultivars Dajti , Katerina, Apache and Krajlica , that are characterized with low FN values and high Fusarium infection, which predict high α-amylase content. The coefficient of correlation between C2 and the disease index is r= -0.770 which means that the more damaged the grain is, the lower the protein quality. The effect of starch, gel formation and retro gradation was reflected by C3, C4 and C5 values. As predicted from the values of FN, the values of C3 and C4 were worst for the cultivars with a high degree of infection (r=-0.748).The dough with low values of C3, C4, C5 parameters, is usually stickier and has poor baking quality [17]. All cultivars analyzed in this study are not characterized by high C4 values (0.45 – 1.56Nm). Such behavior could be explained by the presence of a high quantity of damaged starch granulas because of their easier degradation, which is additionally stimulated by the presence of Fusarium infection. According to the other study [45], it was found that the C5 value had a significantly higher correlation with the saccharified starch content, and those values should be within the limits 2.39Nm, 2.73 Nm and up to 3.16 Nm for a flour with good baking quality. In the following study the C5 values range from 0.90 Nm (Dajti) to 2.76 (Apache). Cultivars Lucia, Asul, Mateo, Europa, have the lowest damage degree of starch, whereas the cultivars Dajti, Katerina, Apache, Mia and Krajlica are characterized by the highest damage degree, so we can predict that those are characterized by the poorest baking quality.

TABLE III. MIXOLAB CURVES

Cultivars	Imc	FN	C1(Nm)	C2(Nm)	C3(Nm)	C4(Nm)	C5(Nm)
Dajti	34.3	225	1.15	0.51	1.77	0.64	0.90
Lucia	5.0	259	1.06	0.68	1.93	1.69	2.47
Mia	15.7	257	1.10	0.62	1.69	1.13	1.86
Katerina	11.7	203	1.08	0.57	0.79	0.45	2.01
Europa	6.0	263	1.11	0.73	1.86	1.40	2.40
Asul	5.25	261	1.06	0.69	1.89	1.44	2.40
Krajlica	16.8	260	1.08	0.57	0.79	0.45	2.01
Mateo	4.07	338	1.10	0.70	1.91	1.41	2.78
Apache	29	254	1.05	0.63	0.90	0.36	2.76

H. Glutopeak determination:

From the data obtained from GlutoPeak measurements it can be presumed that the quality of storage proteins has been impaired during the colonization from the fungus. Gluten-forming proteins absorb water and form a network during dough making which gives it the ability to retain gas during the baking process and results in an elastic crumb structure [23, 24]. Fusarium produces proteases that damage storage proteins [25]. As we can see from the results shown on table 4 cultivars Dajti, Apache, Krajlica with highest infection rate and lowest value for FN didn't give peak. Wet gluten was also lower for these cultivars i.e. Table 1 indicates that .Katerina, Mia, with a mean value for disease index, gives a normal peak, similar to Europa , Mateo and Asul with minimum disease indices. The analysis of hierarchic classification (HFC) to obtain the dendogram of similarity (Fig 2) was performed using the methods of Euclidian distance for the variables Imc (%), Ash(%), K-SDS, Proteins (%) and wet gluten. By incision of the dendogram, the nine cultivars of soft wheat were categorized in four clusters. Dajti(1) and Apache (9) are in the first cluster with a similar disease index and similarity of 78%. In the second cluster Lucia (2), Europa(5) , Asul (6) and Mateo (8) also had a close disease index with one another and similarity level (Europa and Asul 91.5%, Europa and Mateo 85% and Lucia and Europa 74.7%). Mia (3) and Katerina (4) are in the third cluster with a disease index of 15.7 and 11.7 and a level of similarity of 82%. Krajlica (7) was in the fourth cluster with imc 16.8, and it has the closest similarity level with Mia 61.4. It can be detected that same classes are determinate from behavior of the same cultivars towards disease index.

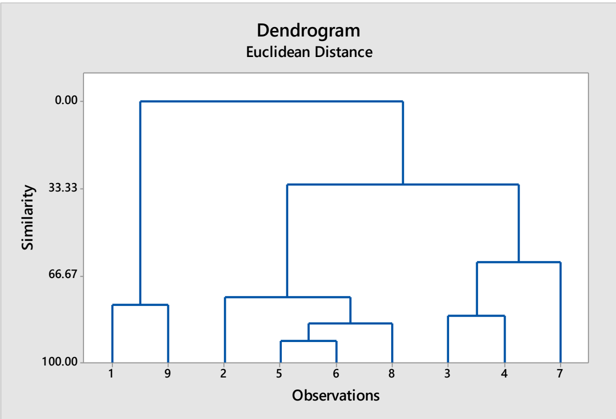

Fig 2. *Cluster Analysis of Observations: Ash(%), TKW (1000 Kernels weight) , Imc (%), K-SDS, Proteins(%), WG(%).*

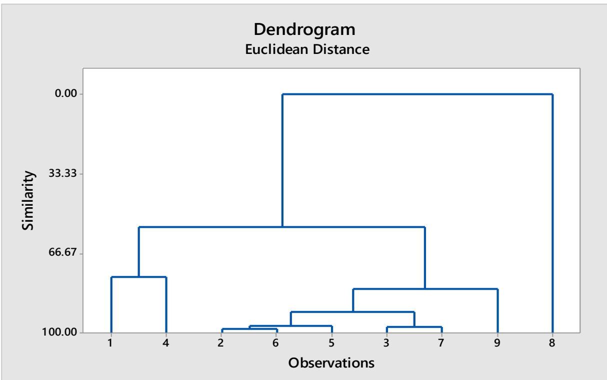

Fig. 3. *Cluster Analysis of Observations: Imc, FN, C1(Nm), C2(Nm), C3(Nm), C4(Nm), C5(Nm).*

The analysis of hierarchic classification (HFC) in order to obtain the dendogram of similarity (Fig 3) was performed using the methods of Euclidian distance for the variables Imc (%),FN, C1(Nm), C2(Nm), C3(Nm), C4(Nm), C5(Nm) . By incision of the dendogram, the nine cultivars of soft wheat were categorized in four clusters. The cultivars Dajti (1) and Katerina (4) with highest value for Imc and similarity level 76% were categorized in the first cluster. In the second cluster we have Lucia (2) and Asul (6) , Europa(5), Mia (3) and Krajlica (7) respectively 5, 4.07, 6 and 4.07 with 16.8 % level of similarity (Lucia with Asul, 98.5%, Lucia and Europa with a similarity level of 96.9% ; Mia and Krajlica with 97.5%, while Lucia and Mia with a similarity level of 91.1%. In the third cluster was Apache with 81.6% similarity with Lucia and in the fourth was Mateo (8) with the disease index of 4.07. In this case the disease index is not determinative for other parameters as it is in the first dendogram.

IV. CONCLUSION

The results obtained from the following experiment shows that not all the analyzed traits behave the same way with the increase of infestation in kernels. In the cultivars with a high infection degree an increased of protein content but a decrease of the wet gluten (Dajti and Apache) was observed .Thousand kernel weight and falling number was considerably lower for damaged grain. High correlations were found between Mixolab characteristics, disease index and falling number .The coefficient of correlation between C2 and the disease index was determined to be r = - 0.770. From the study it was observed that FN, C3 and C4 are lower in the cultivars with higher degrees of infection which predicts that the protein quality in those cultivars is worst.

From the data of GlutoPeak measurements it was presume that the quality of storage proteins has been impaired during the colonization form the fungus. Cultivars Dajti, Apache and Krajlica with the highest infection rate and lowest value for falling number didn't give peak. According to the data obtained from this experiment it can be suggested that an increasing intensity of *Fusarium* spp. infestation worsened the rheological quality and led to a decrease of most of the evaluated baking parameters, especially Zeleny sedimentation index, FN and protein quality.

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