Effect of moisture on wheat grains lipid patterns and infection with *Fusarium graminearum*

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ABSTRACT

Suitable conditions of temperature and humidity are required to maintain wheat grains quality, but during processing and storage, the grains can be exposed to adverse environmental conditions and presence of infectious fungi. *Fusarium graminearum*, the main causal agent of Fusarium head blight on wheat, affects crop yields and grain quality by alteration of their biochemical components and mycotoxin contamination, which reduces the possibilities of wheat end use and compromises food safety. Lipid degradation by hydrolytic, oxidative and microbial deterioration is the predominant cause of the loss of sensory acceptability, nutritional value and baking quality. The aim of this research was to determine the influence of adverse environmental conditions –as the increasing moisture – on lipid patterns of whole wheat flours contaminated with *F. graminearum* in relation to the infection degree. *In vitro* cultures of *F. graminearum* were carried out on wheat grains under different degrees of relative humidity (11, 50, 75 and 100%) throughout 45 days of incubation at 28 °C. The fungal biomass measured by q-PCR increased proportionally with the humidity. A decrease in the signals of saturated (palmitic and estearic) and unsaturated (oleic, linoleic and linolenic) fatty acids, analyzed as fatty acid methyl esters (FAMEs) by GC–MS, was observed in relation with the humidity and infection degree. The degradation rate of the lipids was high during the first 15 days of incubation, reaching the fatty acids content, values around 20–40% of those found in the control. From that moment on, the rate of degradation was slower or even null. It was observed that in all treatments, the linolenic acid reached the highest degradation ratio in comparison with the other fatty acids, which may be caused by the action of lipoxygenases. The lipase activity and the content of deoxynivalenol were also determinate on the flours. The lipase activity increased until day 25 of incubation reaching twice the initial value. The deoxynivalenol content also increased along incubation while fatty acids decreased. Our results demonstrated that the magnitude in the signal of fatty acids in whole wheat flours varied in relation to the degree of humidity and fungal infection of the grains from which they were obtained. Otherwise, lipids and their oxidation products are related with the pathogenesis and production of mycotoxins. These observations highlight the importance of an adequate manipulation of wheat grains on the processing chain to prevent quality changes and mycotoxins contamination.

1. Introduction

Wheat production is led by the European Union, which produces 20% of the world's wheat, followed by China and India. Latin American countries in general are important producers of wheat in the world,

Argentina being in the eleventh position. At present, Russia retains its position as the first exporter, and Argentina ranks seventh in this category (Agrofy, 2018).

Fusarium head blight (FHB) is among the most severe fungal diseases on small cereals worldwide, since the infection directly affects the

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yield and quality of grains, and can contaminated grain with mycotoxin reducing the wheat end-use (Kreuzberger et al., 2015; Matny, 2015). *Fusarium graminearum* species complex (FGSC) is associated with this disease, *F. graminearum sensu stricto* being the main causal agent reported in Asia, North and South America and Europe (Castañares et al., 2014). In South America, the main mycotoxin associated with FHB is deoxynivalenol (DON) which has several adverse effects on animal and human health. Since current regulations for the international marketing of cereals establish its maximum allowed content on wheat derived products, its presence is monitored in grains and flours (Astolfi et al., 2012; Castañares et al., 2014). During processing and storage, the wheat grains can be exposed to adverse environmental conditions. Suitable conditions of temperature and humidity are required to maintain grain quality, and at the same time to delay deterioration, but sometimes these conditions are not achieved. Although deficient agricultural systems are the most predisposed to have outbreaks of pathogen infection due to the difficulty of achieving controlled conditions, the qualified storage systems may result exposed to this type of situation that reduce quality (Homdork et al., 2000; Nazari et al., 2014). The adverse conditions may produce damage in stored grains and flours which are subject to hydrolytic, oxidative and microbial deterioration, processes highly dependent on environmental humidity (Clayton and Morrison, 1972; Manstretta et al., 2016; Stuper-Szablewska et al., 2014). Moisture content and temperature are the main factors that contribute to mold growth and mycotoxin production (Arya and Parihar, 1981; Atalla et al., 2003; Daftary et al., 1970; Nazari et al., 2014, 2018). Lipid degradation involves a cascade of biochemical changes that lead to reduced flour functionality in addition to a reduction in palatability, nutritional properties and increased acidity (Warwick et al., 1979; Zamora et al., 1991). Lipids are structurally diverse biological molecules, which can be classified as simple or complex if their hydrolysis produces two or more products respectively. The simple lipids include free fatty acids (FFA) which form esters when linked with glycerol forming mono-, di- and triacylglycerols (MAG, DAG and TAG respectively). They differ in chain length and can either be saturated as the palmitic and stearic acids or unsaturated as oleic, linoleic and linolenic acids (Manley, 2000). The lipids are further grouped according to their solubility into polar and non-polar according to their extractability in different solvents. Moreover, wheat flour lipids can be classified into three groups: free, bound and starch-internal lipids. The term bound lipids refers to their association with the starch granule surface or with the interface between starch and gluten protein (Chinma et al., 2015; Gerits et al., 2014; Pareyt et al., 2011).

Lipids are quantitatively minor, but significant compounds of wheat, present in the order of 0.9–3.3% in grains. The majority of polar lipids of a wheat grain are present in the starchy endosperm, and the non-polar lipids are predominantly present in the germ and aleurone tissues, and consist of FFA, MAG, DAG and TAG (Prabhasankar et al., 2000).

Wheat lipids have important functions in breadmaking, regarding the framework that forms while interacting with starch and gluten proteins. The qualitative and quantitative characteristics of the lipids affect the gas cells structure which impact on bread loaf volume and crumb structure (Gerits et al., 2015; Pareyt et al., 2011). Moreover, the lipidic degradation compounds are the major culprits in the loss of sensory acceptability, nutritional value and baking quality (Goesaert et al., 2005).

The lipid degradation begins by hydrolytic rancidity, which can be followed by oxidative rancidity. The lipase (EC 3.1.1.3) activity is responsible for the hydrolytic rancidity, releasing non-esterified fatty acids from acylglycerol. In wheat, lipase activity is mostly located in the bran fraction of the grain (Doblado-Maldonado et al., 2012, 2013; Poudel and Rose, 2018). The unsaturated FFA released by lipases can be degraded by enzymatic oxidation or by autoxidation. The enzymatic oxidation occurs through the action of lipoxygenases (LOX) (EC 1.13.11.12). LOXs are a group of isoenzymes (L-1, L-2, L-3) located in

the germ and bran of the wheat grain (Manna et al., 1998; De Simone et al., 2010). Both oxidation processes involve addition of oxygen to polyunsaturated fatty acids, forming hydroperoxides which lead to smaller volatile compounds (*e.g.* hexanal, 2-hexenal, octanal, trans-2-nonal, 2-nonenal, 2,6-nonadienal, *etc.*, Gao and Kolomiets, 2009) through redox reactions, isomerization, chain scission, among others (Doblado-Maldonado et al., 2012; Fierens et al., 2015; Stuper-Szablewska et al., 2014). In particular, plants 9-LOX's and 13-LOX's are involved in the degradation of linoleic and linolenic acid. It is remarkable that plant lipids and their LOX's derived products act as signals to modulate fungal developmental, sporulation and biosynthesis of mycotoxins according to Gao et al. (2007) and Gao and Kolomiets (2009). Moreover, it has been demonstrated that lipases can also be produced by *F. graminearum* during wheat infection, and could contribute to the hydrolysis of triglycerides in wheat grain (Gaillardin, 2010; Subramoni et al., 2010). Lipases together with mycotoxin shave been considered as virulence factors of the pathogen in wheat infection (Ilgen et al., 2008; Voigt et al., 2005).

Since lipid degradation is one of the main causes of quality loss and reduction of the wheat end use, this research aimed to determine the influence of adverse environmental conditions –as the increasing moisture and fungal infection– on lipid patterns of whole wheat flours obtained from grains contaminated with *F. graminearum*.

2. Materials and methods

2.1. Sample preparation and treatments

Four relative humidity (HR) conditions were assayed on samples of wheat in order to analyze the effect on their lipids. The employed grains were previously sterilized by irradiation with 10–12 kGrays of gamma energy (CNEA, Argentina). The tests were carried out from four groups of wheat grains with different moisture, which were achieved by mixing hydrated grains with dry grains in the following proportions: 11/89%, 50/50%, 75/25% and 100/0%. The wheat grains were placed on Petri dishes forming a monolayer (20 g) and stored inside hermetic plastic boxes with the corresponding saturated salt solutions to achieve the relative humidity of 11, 50, 75, 100% (Rockland, 1960). The 100% HR was achieved with water and a drop of glycerol (Palazzini et al., 2007). Then, the Petri dishes were inoculated with a 4-mm-diameter agar disk taken from the margin of a 7-day-old growing colony of a highly aggressive *F. graminearum* isolate on potato dextrose agar (PDA, DIFCO, USA) (Ortega et al., 2018). The hermetic boxes were placed in a culture chamber at 28 °C. At 5, 10, 15, 20, 25, 30 and 45 days of incubation, the cultures of two Petri dishes of each treatment were dried in a forced air oven and finely ground with an electric grinder FW 100 (Arcano, Argentina). The flours obtained were used for the subsequent evaluations. The assay was carried out by independent duplicates.

2.2. Quantification of fungal biomass by q-PCR

The growth of *F. graminearum* was followed by the quantification of its DNA by a specific qPCR. For each trial 1 g of flour was weighed in a sterile bag and homogenized with 10 mL of sterile water. The total DNA was extracted from 2 mL of each homogenate using the ZR Fungal/Bacterial DNA MiniPrep™ (ZymoResearch, USA). The amount of DNA of *F. graminearum* in each sample was quantified by duplicate by real-time PCR using the FgramB379 and FgramB411 primers (Nicolaisen et al., 2009). The SYBR Green PCR amplification mixture was performed in 10 µL containing 5 µL of 2× Rotor-Gene™ SYBR®Green PCR Kit (Qiagen), 0.5 µL of each primer (25 µM), 2 µL of the sample and 2 µL of deionized water. Real time PCR reactions were performed in a Rotor-Gene™ 6000, Corbett Life Science. The PCR protocol consisted of 2 min at 98 °C, 40 cycles of 98 °C for 5 s and 62 °C for 10 s. Following the final amplification cycle, a dissociation curve analysis was carried out by continuously measuring the fluorescence when heating from 55 to 95 °C

at the rate of 0.5 °C per second, in order to confirm the specificity of the amplification. A standard curve was generated by duplicate analysis of different amounts of DNA from a *F. graminearum* isolate, ranging from 1.9 to 1.9×10^{-5} ng per reaction tube. Fungal DNA was extracted directly from the mycelium obtained from a 48 h-old culture in PDA at 28 °C with the kit mentioned above. The double stranded DNA concentration in the obtained solution was determined using Qubitds DNA HS Assay Kit (Invitrogen, USA) in a Qubit Fluorometer (Invitrogen, USA). The mean cycle threshold (Ct) values were plotted against log of the corresponding DNA amount in the reaction tube. PCR efficiency was calculated from the slope of the standard curve with the formula: Efficiency = $[10(-1/\text{slope})] - 1$ (Dorak, 2007). Detection limit and dynamic range were set in the linear range.

In each case Ct values obtained from the samples were interpolated in the standard curve to determine the amount of *F. graminearum* DNA present in each reaction tube. Data analysis was carried out in Rotor-Gene 6000™ cycler software (Quiagen, Venlo, Netherlands).

Total DNA from uninoculated samples was extracted and analyzed by qPCR as described above to determine if there were interferences in *F. graminearum* quantification due to the matrix.

2.3. Fatty acids analysis by gas chromatography-mass spectrometry

The lipid extraction from the flours was carried out according to Marchetti et al. (2018) using a microwave oven MARS (CEM Corp, USA). For each treatment, 0.5 g of flour were weighed into a flat-bottomed flask, mixed with 1 mL of extraction solvent (Cl₃CH:EtOH·2:1) and extracted in a microwave oven for 3 min at 400 W. The mixes were transferred to a 2 mL microtube and centrifuged at 10000 rpm for 3 min. The supernatants were recovered and evaporated with a stream of N₂. The obtained pellets were resuspended in 500 µL of heptane. Then, the basic methyl-esterification was achieved by adding 200 µL of a solution 2 N of KOH in MetOH to each treatment and incubating for 20 min in agitation by vortex at room temperature. The reactions were stopped by adding an excess of NaHSO₄ and centrifuged at 10000 rpm for 3 min, thus an upper phase of fatty acid methyl ester (FAME) was obtained for posterior analysis.

FAMEs content of each extraction was assayed by duplicate employing a Shimadzu GC–MS model QP-2010 Ultra and Hewlett Packard's (HP) model HP-6890. An AT-WAX (Alltech Associates, Inc.) capillary column 30 m in length and an internal diameter of 0.25 mm coated with polyethylene glycol (0.25 µm film thickness) was used. Hydrogen (> 99.999%) was used as carrier gas at constant flow rate of 1.4 mL min⁻¹. MS interphase and ion source temperatures were 270 °C and 220 °C, respectively. The temperatures of the injector and the detector were set at 250 °C and 270 °C, respectively. The analysis was performed using full MS scan from *m/z* 50 to 400. A solvent delay of 3 min was used. Injection volume of 1 µL and a split ratio of 50:1 were used as part of the GC–MS analysis method (Marchetti et al., 2018).

2.4. Lipase activity

The lipase activity was carried out according to Rose and Pike (2006). One gram of each flour sample was defatted three times with 10 mL of hexane for 30 min on an orbital shaker at 160 rpm. The defatted flour was mixed with 0.2 mL of distilled water and 0.6 mL of olive oil and incubated at 40 °C for 4 h. The lipids were extracted three times with 5 mL of hexane. Each time the sample was shaken and centrifuged at 2300 rpm for 3 min. The hexane extracts were recovered and combined, evaporated on a rotary vacuum evaporator at 40 °C and redissolved in 4 mL of isooctane. Free fatty acids (FFA) were quantified by duplicate according to the method of Kwon and Rhee (1986). One milliliter of 5% cupric acetate solution (adjusted to pH 6.1 with pyridine) was added and shaken vigorously for 1 min. The tubes were centrifuged at 2300 rpm for 1 min and the supernatants were read in a T60 spectrophotometer at 715 nm (PG Instruments, UK).

Lipase activity was expressed as units/g (U/g), where 1 U was defined as the micromoles of FA liberated per hour according to Rose and Pike (2006), as follows:

$$U/g = 1000 \left[(4 + v)(Af - Ai)/\varepsilon t l s \right]$$

where 1000 = conversion factor from mol/L to µequiv/mL; 4 = volume of isooctane used to redissolve lipids (mL); v = volume of olive oil added (mL); Af = absorbance of sample after incubation at 715 nm; Ai = absorbance of blank at 715 nm; ε = molar absorptivity of oleic acid at 715 nm ($M^{-1} \cdot cm^{-1}$); t = incubation time (h); l = path length (1 cm for a standard cuvette) and s = sample weight (g).

2.5. DON analysis

DON was extracted from the flours with 125 mL of acetonitrile:acetylacetate:water (50:41:9) with agitation for 1 h at 300 rpm. The clean-up was performed with a column packed with charcoal:alumina:celite (0.7,0.5:0.3) and dried in Rotavap®. Gas chromatography, with 63Ni electron capture detection Shimadzu Model GC17 equipped with RX-5MS capillary column (25 m × 0.2 mm id), were used to detect and quantify DON. The detection limit was 0.02 µg g⁻¹. DON standard was purchased from SIGMA Chemical Company (St Louis, MO, USA). The presence of this compound was confirmed by Gas Chromatography–Mass spectrometer system (GC–MS QP 5050A, Shimadzu®) with Electron Impact (EI) mode (70 eV) as described by Alvarez et al. (2009). The samples were analyzed by duplicate.

2.6. Statistical analysis

The statistical analysis of the data (ANOVA test) was carried out employing STATISTICA 7 software (StatSoft).

3. Results and discussion

In this work, we have analyzed the influence of adverse environmental conditions during wheat grain storage on biochemical components related to flour quality. Since lipid degradation causes loss in flour quality, we studied the effect of increasing humidity and infection degree by *Fusarium graminearum* on lipid patterns of flours obtained from grains stored in those conditions. Our results demonstrated that both saturated and unsaturated fatty acids analyzed by GC–MS decreased during storage in the assayed conditions. As infection complementary measurements, the lipase activity and DON content were assayed on the flours.

3.1. Effect of moisture on F. graminearum growth

F. graminearum growth on grains was assessed by quantification of its DNA by a specific qPCR, as a measure of its fungal biomass. The qPCR method used in this work was based on the one developed by Nicolaisen et al. (2009). In the conditions of the present work an efficiency of 99% was obtained, which was calculated from the slope of the standard curve (R^2 = 0.99636). Melting curve analysis showed that in every case there was only one amplicon with a melting temperature of 80.3 °C, as expected. No interferences of the matrix were observed since reactions without *F. graminearum* DNA did not generate amplification products. In the conditions of our work, the quantification limit of the qPCR method was 0.19 pg, corresponding to approximately 5 *F. graminearum* haploid genomes (Rossi et al., 2007). This limit resulted similar to the one reported by Nicolaisen et al. (2009) (0.1 pg) and more sensitive than the obtained by Terzi et al. (2007) (4 pg) with different primers. According to our limit the minimum concentration of *F. graminearum* DNA that could be quantified in wheat flour, using the present method was 0.25 ng/g.

At 11% RH *F. graminearum* DNA was below the detection limit of the method all over the incubation period. At 50% RH the pathogen DNA
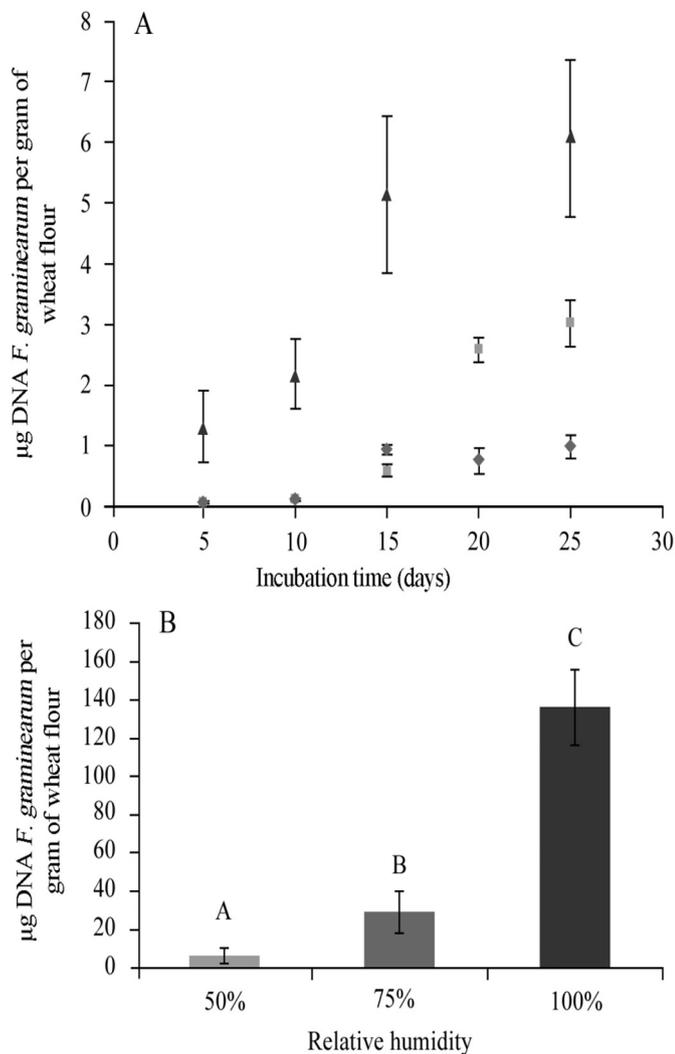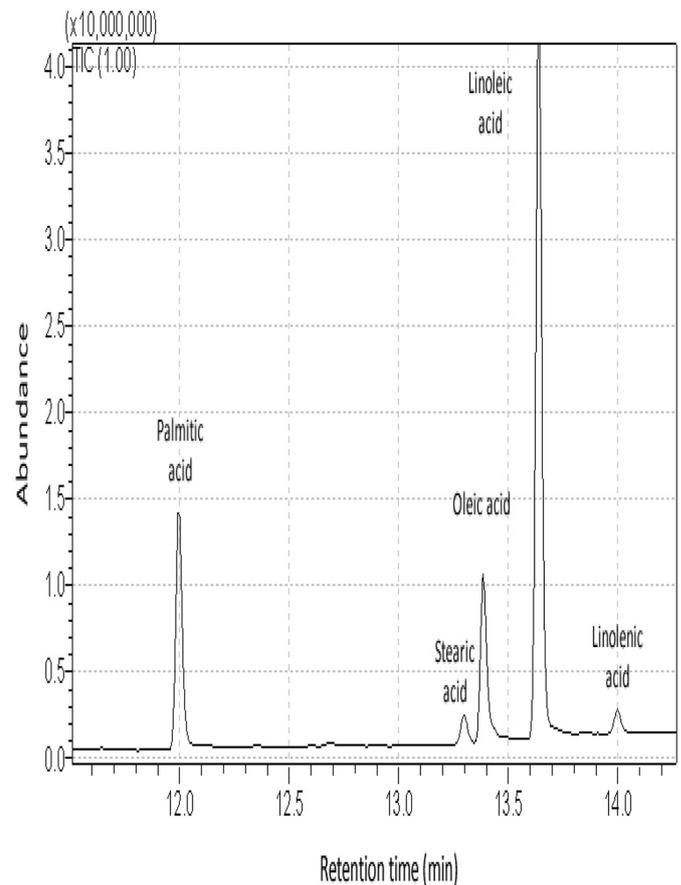
Fig. 1. Growth of *Fusarium graminearum* cultivated on wheat grains at different relative humidity by qPCR. A) Incubation time: 5 to 25 d. Relative humidity (RH): 100% (▲); 75% (■); 50% (◆). B) Incubation time 45 d.

Fig. 2. Fatty acid methyl esters pattern of whole wheat flours contaminated with *Fusarium graminearum* obtained by GC–MS. The identification of Palmitic acid (C16:0), Stearic acid (C18:0), Oleic acid (C18:1), Linoleic acid (C18:2) and Linolenic acid (C18:3) was carried out matched with the NIST library patterns.

reached the threshold of detection at day 10. Growth curves of the fungal pathogen on wheat grains at different levels of moisture over the first 25 days of incubation are shown in Fig. 1A. The fungal biomass increased over the incubation time proportionally with the humidity level. After 5 days of incubation the fungal biomass at 100% RH was significantly different from the biomass obtained in the other treatments. However, significant differences among the treatments 50% RH and 75% RH were not found until day 20. At day 45 a burst in *F. graminearum* DNA content was observed in the three treatments (50, 75 and 100% RH), which could be due to sporulation of the fungal pathogen. Fig. 1B shows the maximum DNA content at different % RH observed after 45 days of incubation, which were significantly different in all the treatments. These results emphasize the importance of moisture control to prevent the development of fungal contamination on stored wheat grains.

3.2. Changes of fatty acid methyl esters by GC–MS

The samples obtained from the lipid extraction of infected wheat flours for each treatment and the respective controls were injected, after derivatization on GC–MS equipment for their analysis. The effect of humidity and *F. graminearum* growth on fatty acids pattern through time in whole wheat flours was observed by changes of the intensity of
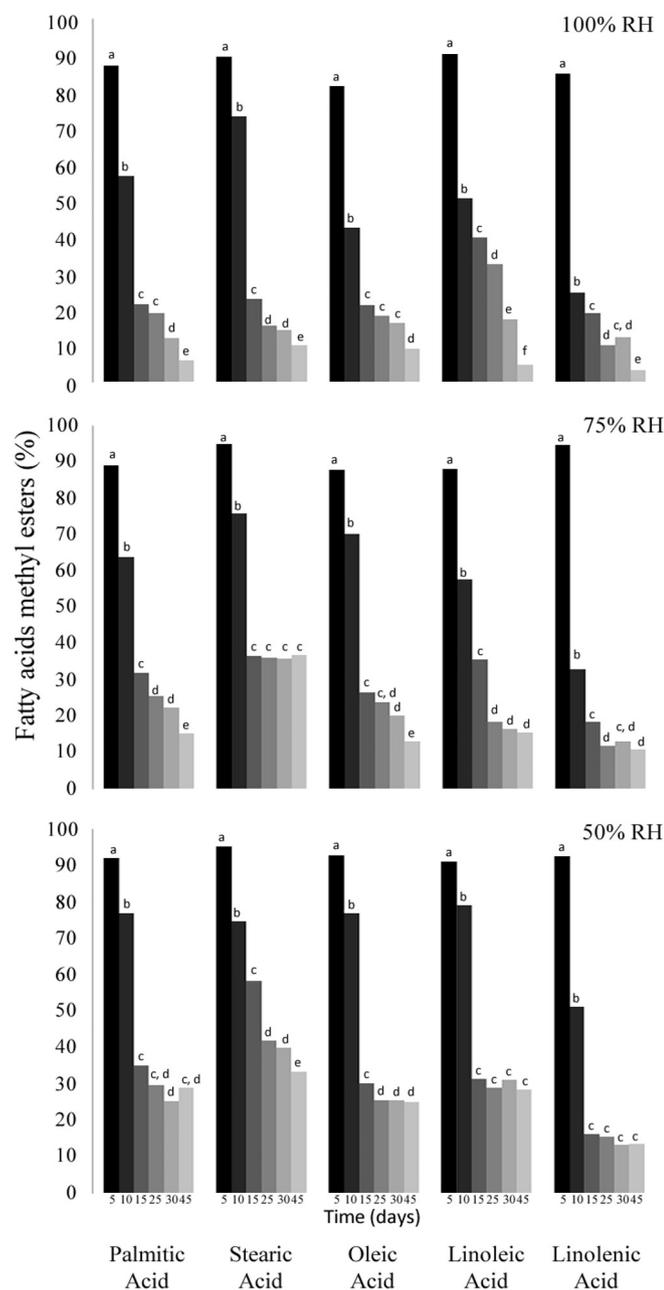
GC–MS signals. In Fig. 2 it is shown a representative profile of FAMEs by GC–MS, in which the observed signals correspond to palmitic, estearic, oleic, linoleic and linolenic acids according to the "*m/z*" NIST pattern library. The relative content of the different fatty acids observed in the control samples were in agreement with previous researches on wheat (Chinma et al., 2015; Durante et al., 2012; Sjövall et al., 2000).

The integrated areas of the obtained FAME peaks were employed to compare the fatty acid content in all samples. In comparison with the uncontaminated control wheat grains, a decrease of all FAME signals was observed through time in relation with the relative humidity in the analyzed treatments (Fig. 3). After 15 days of incubation, the content of each fatty acid in every sample represented around 20–40% of the value in control, with exception of the stearic acid in 50% RH which was 58%. From that moment on, the rate of degradation was slower and even null. The lowest levels of fatty acids were obtained in samples with 100% RH, in which FAME content was below 10% of the original value after 45 days of incubation. It was observed that in all treatments, the linolenic acid reached the highest degradation ratio in comparison with the other fatty acids.

The most abundant lipids in wheat are fatty acid esters of glycerol, mainly triacylglicerides which are relatively stable in sound grains (Morrison, 1994). However, in certain storage conditions and in presence of molds, those lipids can be degraded. At first, fungal or grain lipases (the last acting mainly in flours or damaged grains) cleave ester bounds of TAG producing an increment of the respective fatty acids (Wang and Flores, 1999), which can be further degraded by oxidation. The β-oxidation of unesterified fatty acids is the first step of their incorporation into the respiratory chain and oxidative phosphorylation (Kunau et al., 1995) acting as energy and carbon sources for fungal

Fig. 3. Effect of the relative humidity on the fatty acid methyl esters (FAME's) content through culture time of *Fusarium graminearum* infected wheat grains measured by GC–MS. The FAME's percentage values were obtained considering the signals on the control wheat flour as 100%. Different letters represent significant differences.

Table 1
Measurements on whole wheat flours infected with *F. graminearum* in treatment 50% RH.

Incubation days	DON	L A	Fatty acids (%)				
	(ppm)	(U/g)	A	B	C	D	E
Control	–	2.07[a]	100.00	100.00	100.00	100.00	100.00
5	0.94[a]	1.98[a]	92.21	95.26	92.93	91.22	92.54
10	13.24[a,b]	2.70[b]	76.94	74.80	76.94	79.18	51.29
15	5.12[a]	2.61[b]	34.98	58.30	30.24	31.45	16.22
20	1.79[a]	3.70[c,d]	21.85	51.18	27.19	28.6	14.34
25	21.20[b,c]	4.16[e]	29.60	41.98	25.61	29.05	15.56
30	29.64[c]	3.53[c]	25.34	39.95	25.42	31.15	13.28
45	2.54[a]	3.82[d]	28.84	33.25	24.92	28.37	13.48

DON = Deoxynivalenol; L A = Lipase activity; A = Palmitic acid; B = Stearic acid; C = Oleic acid; D = Linoleic acid; E = Linolenic acid. Different letters represent significant differences.

increasing moisture content (Arya and Parihar, 1981; Dahle, 1965), which is in concordance with the decrease on the content of linoleic and linolenic acids at high moisture level as in the present study.

Since on the 50% RH treatment the fatty acids decreased more gradually, it was decided to continue the posterior analysis of the infection –lipase and mycotoxin measurements– on this condition.

3.3. Lipase activity

The lipase enzyme, responsible for the hydrolytic rancidity, acts in the first step of the metabolic pathway of fatty acids and their incorporation as nutrients by the fungus. For this reason, the lipase activity was measured on the infected flours, showing increase of the activity until day 25 of incubation, and reaching twice the initial value (Table 1). It can be observed that, in the control flour, the endogenous lipase activity detected was around the reported values (Rose and Pike, 2006). The increase of this enzymatic activity as a result of *Fusarium* infection is in concordance with those studies that considered it as virulence factor during infection (Gaillardin, 2010; Ilgen et al., 2008; Subramoni et al., 2010; Voigt et al., 2005). Feng et al. (2005) found that *Fusarium* lipases are induced by the presence of long chain extracellular lipids, which could explain the relative enzymatic decrease observed in the present study.

4. DON analysis

The DON content was analyzed in our research since this mycotoxin is the most commonly associated with *F. graminearum* infection and its presence is monitored before international marketing of cereals (Astolfi et al., 2012; Castañares et al., 2014; Umpiérrez-Failache et al., 2013). Limit of DON allowed has been fixed in 1 and 2 ppm for wheat flours and unprocessed grains respectively, according to the Codex Alimentarius Commission (2016), whereas the European Union fixed these values in 0.75 and 1.25 ppm respectively (Kreuzberger et al., 2015). As is observed in Table 1, the DON content was below allowed limit only until the fifth day of incubation, then showing a significant increase at days 25 and 30 which was in agreement with the decrease on the rate of degradation in the lipid fraction.

After 25 and 30 days of incubation it was also observed decreasing values of fatty acids and increasing lipase values. DON and lipase production are important virulence factors in wheat and are essential for plant infection (Sella et al., 2014).

In conclusion, adverse environmental conditions of humidity and *Fusarium* infection demonstrated the high susceptibility of the wheat flours fatty acids to degradation. Since storage systems may be exposed to adverse conditions and the lipids and its oxidation products act as signals in the pathogenesis and the production of mycotoxins, this research highlights the importance of an adequate manipulation of the

development (Poirier et al., 2006). The oxidation process can also be consequence of the autoxidation (Robards et al., 1988; Shahidi and Wanasundara, 2002) or caused by effect of lipoxygenases (LOX) which oxidize the polyunsatured fatty acids (as linoleic and linolenic acid) forming lipid hydro peroxides. The LOX enzymes are mainly studied in plants such as wheat, in which they are present on bran and germen of the grain (Doblado-Maldonado et al., 2012) and can have a role in lipid degradation in wheat flours. Only a few reports of LOX in fungi have been published, *i.e. Saprolegnia parasitica*, *Malessezia furfur*, *Psallioata bispora*, *Gaeumannomyces graminis* (Oliw, 2002), *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Pseudomonas* spp. (Stuper-Szablewska et al., 2014) and in *Fusarium* genus (Bisakowski et al., 1995). Furthermore, the rate of activity of these enzymes has been reported to increase with

wheat grains on the processing chain.

Declaration of Competing Interest

None.

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