Baking quality of wheat flour affected by cereal aphids

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Summary

Flour from grains originating from plants infected artificially with cereal aphids were analyzed for glutenin and gliadin and total protein content, using Size Exclusion HPLC. Wheat plants were caged at the beginning of stem elongation. Cages were treated with 0.1 % methyl parathion. One week later, the caged plants were artificially infected with 5 aptera individuals of *Metopolophium dirhodum, Diuraphis noxia, Sitobion avenae* and *Rhopalosiphum padi.* It was found that aphid infection had significant effect on the glutenin and gliadin content, the total protein content and the gliadin/glutenin ratio. Both the glutenin and gliadin content was significantly higher in the seeds harvested from aphid infected plants. However, the gliadin/glutenin ratio was significantly lower in wheat flour prepared from aphid infected plants than in those from uninfected control. The most significant decrease in gliadin/glutenin ratio was caused by *M. dirhodum, D. noxia, S. avenae* infection followed by *R. padi* at high-abundance. As the gliadin/glutenin ratio was significantly lower in flours made from aphid infected wheat seeds, it may be suggested, that aphid feeding results in decreased bread making quality of wheat flour.

Keywords: Cereal aphids; *Metopolophium dirhodum; Diuraphis noxia; Sitobion avenae; Rhopalosiphum padi*; SE-HPLC; Glutenin; Gliadin; Gliadin/glutenin ratio; Total proteins

Introduction

Aphids are most abundant in the temperate region where main cereal growing areas are situated, therefore cereals from all over the world are attacked by aphids. Apart from direct feeding damage (sucking of plant sap) aphids act as vectors of phytopathogenic viruses which cause further quantitative and qualitative damages to these plant (Hoffman and Kolb, 1997). In terms of yield losses resulting from cereal aphid feeding on wheat, *Schizaphis graminum* Rondani and *Rhopalosiphum padi* L. are reported to be more damaging at similar population densities than *Sitobion avenae* F. (Kieckhefer and Kantack, 1988). Kuroli and Németh (1987) reported 33-65 % yield loss of winter wheat due to autumn infestation of *R. padi.* The Russian wheat aphid *Diuraphis noxia* Kurdjumov was described to cause significantly greater yield loss than *S graminum,* (Gellner et al., 1990). As a result of quality control measures in use since 1860, Hungarian wheat is notable for its overall good features (Pollhamer, 1981).

Bread-making quality of wheat flour is determined primarily by its protein content and composition as it was summarized by Mac Ritchie (1984), and much effort has been made to elucidate which protein constituents are responsible for specific quality differences. Gluten proteins (glutenins, gliadins) are the prime factors governing wheat properties (Finney, 1943; Mac Ritchie, 1978). Allelic composition of the gliadin- and glutenin loci as well as the absolute amount and/or the relative ratio of gliadins to glutenins are very important in dough making and in determining baking quality.

Originally, the gluten proteins were divided into two main classes according to their solubility in 70% ethanol; soluble proteins were classified as gliadins and insoluble proteins as glutenins (Osborne, 1907). The distinction between solubility classes is not sharp enough and the definition of these classes based on their molecular size seems to be a better approach.

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Polymeric glutenins are proteins formed from glutenin subunits through intermolecular disulphide bonds and they are larger than 100 kDa. Gliadins are between 100 and 25 kDa, while proteins smaller than 25 kDa were defined as albumin and globulin (Meredith and Wren, 1966; Bushuk and Wrigley, 1971). The subunits of glutenins can be divided into two groups: high molecular weight (HMW) (Mac Ritchie et al. 1990) and low molecular weight (LMW) glutenin subunits (Shewry et al. 1992). According to an other and widely accepted approach the wheat gluten proteins are distributed into three main groups: the S(sulphur)-rich prolamins, the S-poor prolamins and the High Molecular Weight (HMW) prolamins (Shewry and Tatham, 1990).

Size-exclusion High Performance Liquid Chromatography (SE-HPLC) has been successively used for the study of cereal storage proteins, particularly in wheat. This methodology reliably and reproducibly separates the three main classes of wheat storage proteins: glutenins, gliadins and albumins+globulins (Singh et al., 1990; Batey et al., 1991; Gupta et al., 1993). It is quite evident that the above qualities have been studied extensively by food scientists and plant breeders, and results in this area are well documented (MacRitchie, 1978; Shewry and Tatham, 1990). Infection by cereal aphids is also in the focus of interest in the plant protection field (Rabe et al., 1989).

In the present publication we compare the glutenin, gliadin and albumin+globulin contents of flours from plants artificially infected with cereal aphids indigenous to Hungary with those of flours from plants infected with *D. noxia*. Macroscopic features and evaluation of quality markers were used to identify qualitative and quantitative changes related to dough making and to the baking quality of different wheat flours originating from respective plants damaged by aphid feeding. The results, obtained by using SE-HPLC, allowed calculation of gliadin/glutenin ratios, comparison of total protein contents.

Materials and methods

Artificial aphid infection

Winter wheat (*Triticum aestivum* L.) cultivar 'MV 17' (Hungary) was sown on 28th October 2000 at a seed rate of 220 kg ha at the experimental plot of the Plant Protection Institute of the Hungarian Academy of Sciences, Nagykovácsi, Hungary. Three hundred thirty plants were selected randomly and caged individually, using a fine mesh cloth to block aphid emigration and immigration, on 25th April 2001 at a growth stage of 25-30 tillering and beginning of stem elongation. Plants after caging were sprayed with 0.1 % methyl-parathion to kill aphids responsible for natural infection, then cages were closed. A week later 60 caged plants were artificially infected with 5 aptera individuals of *M. dirhodum*, *D. noxia*, *S. avenae*, respectively. Hundred-twenty caged plants were infected with *Rhopalosiphum padi* L., and 30 caged plants were left uninfected. Half of the aphid-infected cages were used for aphid population assessment and the other half was used for quality studies. One month following caging *R. padi* infected cages were opened and were labeled high or low according to the *R. padi* density. Six replicates from each category: *M. dirhodum*, *D. noxia*, *S. avenae* and *R. padi* high and low densities and uninfected control caged plants were kept for grain harvest. One replicate consisted of 5 cages.

Aphid population assessment

The plants were sampled destructively at weekly intervals over the six weeks from 11th June 2001 (GS 39, flag leaf collar just visible) until 9th July 2001 (GS 87, hard dough, ripening). Five plants from *M. dirhodum*, *D. noxia*, *S. avenae* and *R. padi* infected cages were sampled at each sampling date. Plants were transferred to Berlese funnels for 5 days to collect aphids. Aphids were extracted from the funnels into 70 % aqueous ethanol and the numbers of different species were counted.

Harvesting for flour quality studies
Heads from *M. dirhodum, D. noxia, R. padi* high-and low-densities and *S. avenae*-infected and uninfected cages (six replicates as above) were collected separately at GS 93 (caryopsis loosening in daytime). Grain from each cage was kept together as one sample for further analyses, yielding a total of 180 samples (each containing 10 heads).

Processing of grain and milling
Following harvesting, length of the heads was measured, kernel number/head was counted, seeds were removed from heads and 30 randomly selected kernel-weight was measured from each treatment. The seeds from each cage were stored in respective bags and separately milled. An FQC-Micro scale labmill (Technical University, Budapest, Hungary) was used to grind the seeds. Flour was separated with a 150-200 μm sieve and the fraction under 150 μm was used for SE-HPLC.

Quantification of polymeric proteins by SE-HPLC
A modified version of methods described by Singh et al. (1990) and Batey et al. (1991) was used (Basky and Fónagy, 2003) to determine the glutenin, gliadin and albumin+globulin content of the samples by SE-HPLC. From each flour sample (total: 180) the extraction was performed and two parallels were run. The elution profile was divided into three main peaks corresponding to polymeric proteins, containing mainly glutenins, the gliadins and the albumins+globulins, respectively. The effect of aphid infestation on the amount of respective macro-proteins and total proteins was calculated on the basis of the previously deduced concentration value multiplied by a generated kernel weight correction factor (kernel weight(g)/0.033628(g); where the denominator is the average of all measured kernel weights) which is proportional to the kernel weight. The obtained result represents the amount of different protein classes in individual kernels. In this study the respective and total protein integrated peak areas were subjected to statistical analysis.

Statistical analysis
Analysis of variance was used to prove the effect of different aphid species on the length of the heads, kernel number/head, weight of the kernels and the qualitative characters as glutenin, gliadin and the albumin+globulin contents of the flour samples (the average of two parallels were taken), as well as the gliadin/glutenin ratio using the integrated areas under the peaks for the proteins obtained by the SE-HPLC procedure. Tukey HSD test was used for post hoc tests for comparison of mean values. Analyses were made using the STATISTICA 5.5 (2000) program package.

Results

Artificial aphid infection
Aphid population was the highest on 18th June regardless of the aphid species (Fig. 1). *R. padi* formed the most abundant colonies on plants where *R. padi* populations were high, the lowest aphid density was recorded in *R. padi* low density cages. *S. avenae* proved to be the second most abundant species, followed by *M. dirhodum* and *D. noxia.* The mean of *R. padi* individuals on the *R. padi* High labeled plants were 134.6 aphids/tiller on 18th June, followed by 55.6 *S. avenae*/tiller, 35.1 *M. dirhodum*/tiller, 25.0 *D. noxia* individuals/tiller, and the mean value of *R. padi* individuals on the Low labeled plants were 8 aphids/tiller at peak population.

Damage caused by aphids
As demonstrated below, analysis of variance revealed significant effect of the aphid infection on the length of the heads, number of kernels/head, kernel weight and the qualitative characters as glutenin, gliadin, albumin+globulin and the total protein contents of the flour.

Fig. 1. The mean number of aphids per cage on the artificially infested plants.

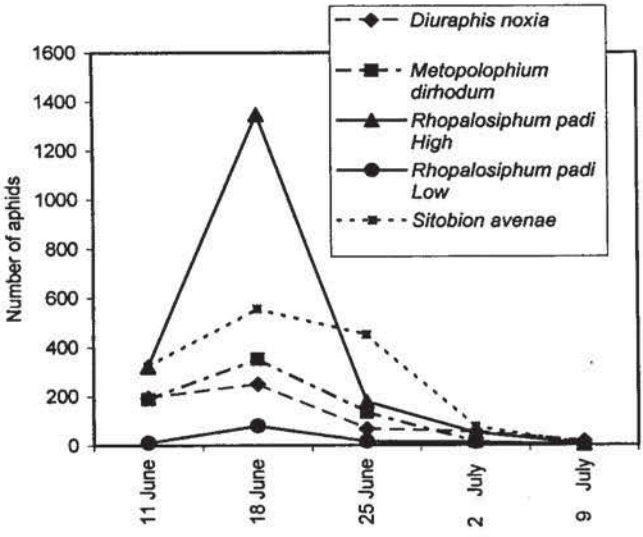
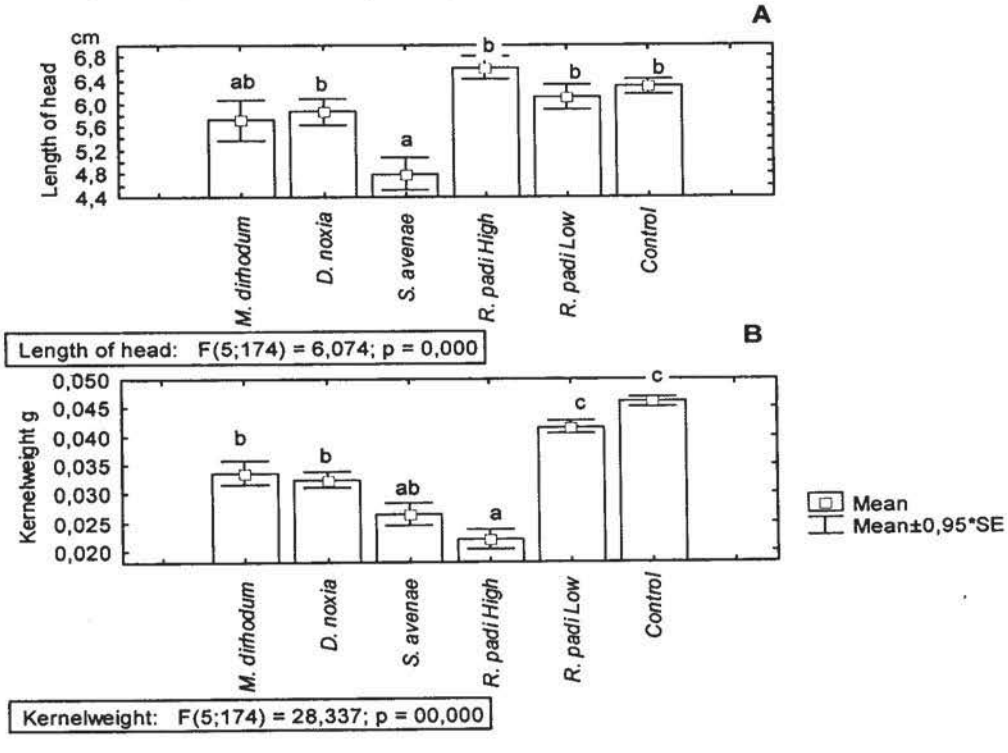


Fig. 2. Mean length of heads and mean kernel weight of uninfected plants and that of infected by *M. dirhodum, D. noxia, S. avenae* and *R. padi* High and Low numbers.



Changes in the quantitative and qualitative characters: Mean head length varied between 2.5 and 10.5 cm, while mean kernel number/head varied between 14 and 65, the mean kernel weight varied between 0.011 and 0.054 g (Fig. 2a). Only *S. avenae* infection resulted in significant decrease in head length. However, apart from *R. padi* Low infection level other aphid species and *R. padi* High-density significantly decreased the weight of individual kernels (Fig. 2b).
The gliadin content of the wheat flour significantly grew due to *D. noxia, S. avenae,* and *R. padi* feeding (Fig. 3a). The glutenin content of the flour also significantly increased in response to aphid feeding damage regardless of the species (Fig. 3b). The gliadin/glutenin ratio, however, significantly decreased due to aphid feeding damage, except when *R. padi* was present on the isolated plants in Low-density (Fig. 3c). Albumin and globulin content of the flour was significantly different from the control when flour was acquired from *M. dirhodum* damaged kernels (Fig. 3d). Total protein content of the flour significantly increased compared to the control in flour originated from kernels damaged by *D. noxia, S. avenae, R. padi* both High- and Low-densities (Fig. 3e).
Qualitative composition in respect to individual kernel gave a somewhat different result, as summarized on Fig. 4, but well correlates with individual kernel weights (Fig.2b).The absolute amount of gliadin was significantly lower in case of *S. avenae* and *R. padi* High, while as for glutenin only *R. padi* High-infestation lowered the amount significantly. Albumin and globulin amount per kernel was significantly lower in all infestation cases. Total protein per kernel also decreased in all cases except in *R. padi* Low-infestation.

Relationship between yield characters: There was significant correlation between the length of the heads and the number of kernels/head, weight of kernels/head and mean kernel weight. There was a close correlation between the mean kernel weight and the weight of kernels/head, glutenin and gliadin content of the flour, gliadin/glutenin ratio and the total protein content of the flour (data not shown). A negative relationship between weight of kernels and glutenin, gliadin and total protein contents of the flour was found.

Discussion

Kieckhefer and Gellner (1992) showed considerable yield decrease (30-40%) caused even by the low autumn infestation of *R. padi.* When *S. avenae* abundance reached 20-30 aphids per plant during milk development quite significant yield decrease was reported (Lee et al., 1981). They also demonstrated that *S. avenae* infestation affected not only yield quantity but bread-making quality of flour, like color, increased nicotinic acid and thiamine content and decreased HMW-glutenin content. Recently Sivri et al. (2004) demonstrated that total proteins extracted from control and bug damaged samples differed in size distribution of the polymeric protein and their glutenin/gliadin ratios. They supposed that bug protease caused dough weakening by degradation of glutenin, presumably by hydrolysis.
During feeding activity the first thing an aphid does after entering a cell with its piercing-sucking mouthpart is to salivate, because it needs to wet and clean its food receptacle and gustatory sensilla of residue from previous sap sampling or feeding (Harris and Harris, 2001). Enzyme containing aphid saliva begins detoxification and digestion of the plant sap before it enters into the aphid alimentary canal. Aphid saliva spreads all over the plant within hours, after aphid feeding begins (Giménez, et al., 1997).
The examination of the studied 1800 heads showed that components of the yield decreasing effect of aphid damage is notable. From the examined cereal aphid species *S. avenae* feeding significantly decreased the length of heads, the number of kernels/head and the weight of kernels/tiller (data not shown). Except in case of the Low-population of *R. padi,* all other species *M. dirhodum, D. noxia, S. avenae* and *R. padi* High-density significantly decreased the weight of individual kernels.

Fig. 3. Effect of aphid infection on the protein content of winter wheat flour. The values on y axis represent the integrated values of HPLC peaks obtained by processing 10 mg of flour by samples (data are mm^2 for A, B, D, E and ratio data for C).

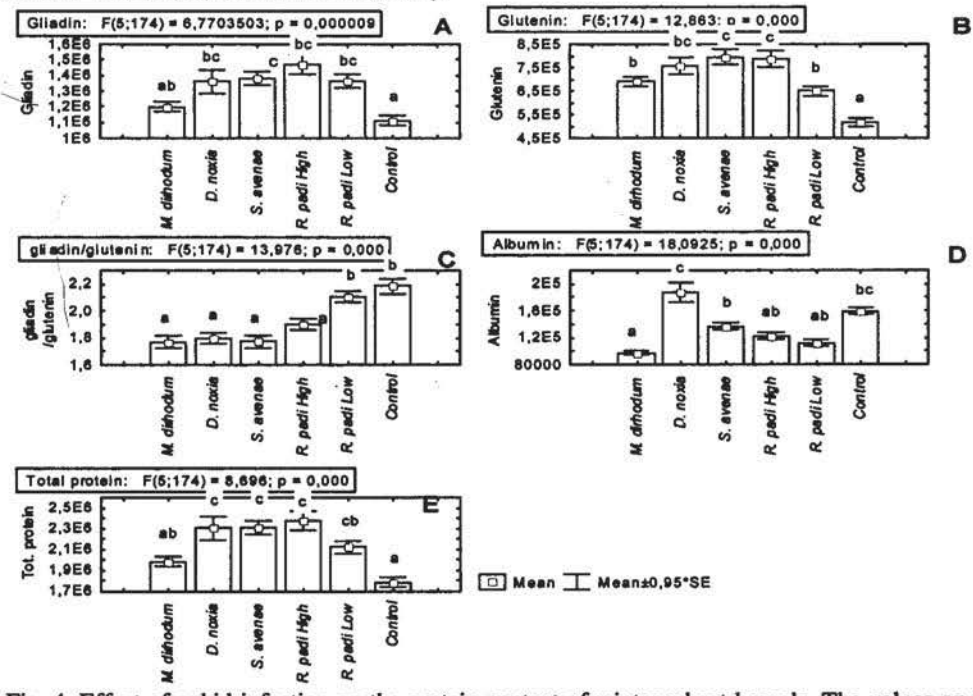
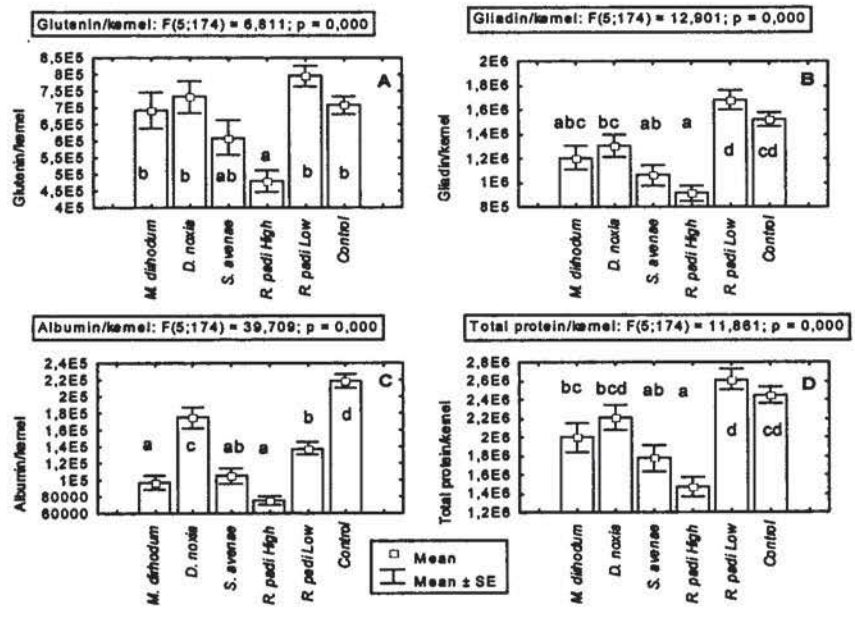


Fig. 4. Effect of aphid infection on the protein content of winter wheat kernels. The values on y axis represent the integrated values of HPLC peaks obtained by processing 10 mg of flour by samples multiplied by the mean kernelweight in the given sample.



It is well known that, glutenin quality and quantity governs mixing requirements and dough strength while gliadin quality and quantity is primarily responsible for dough extensibility. The balance of dough strength and extensibility determine loaf volume (Finney et al., 1982). Therefore, the absolute amount and/or the relative proportion of gliadins to the glutenins are critically important in dough making and determining baking quality.

Cereal aphid species, *D. noxia, S. avenae, R. padi* High- and Low-densities significantly increased gliadin content of the flour. The glutenin content of the flour also grew significantly regardless of the aphid species. In spite of significant glutenin and gliadin content increase due to aphid feeding the gliadin/glutenin ratio decreased notably regardless of the aphid species. These finding are in good correlation with our previous findings from year 2000. where significant decrease in gliadin/glutenin ratio was reported caused by *D. noxia* infection, followed by *R. padi* and then *S. avenae*, respectively (Basky and Fónagy, 2003). On the other hand *M. dirhodum* feeding damage resulted in significant albumin and globulin content decrease as well, while total protein content was significantly higher in flour made from *D. noxia, S. avenae, R. padi* High- and *R. padi* Low-population damaged wheat.

The precipitation was very low during the vegetative period of the wheat, - i.e. spring and summer of year 2001 were very dry -, therefore the kernels were shriveled even in the uninfected control. The negative relationship between weight of kernels and glutenin, gliadin and total protein contents of the flour indicates that proteins were more concentrated in the smaller kernels. Aphid feeding is known to result in a decreased water status of the damaged plants even at sufficient water supply as it was demonstrated by Carberera et al., (1995). Under dry conditions aphid-sucking activity may cause an even greater damage on infected wheat, than under wet weather conditions, causing a significant decline in general quality in respect to bread-making. This is also in agreement with the finding of Blumenthal et al. (1994), who described that heat stress during grain filling leads to important changes in the synthesis of gluten proteins. They reported the reduced synthesis of the HMW subunits of glutenin, and continuing synthesis of other gluten proteins, particularly various gliadin proteins. It is, however, possible that the stress response could be solely due to aphid feeding as was suggested by Békés (pers. comm. 2005).

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