

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF OAT SEEDS NEW LINES IN RELATION TO AGRO-ENVIRONMENTAL FACTORS IN 1997-1999*

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Abstract. Four spring oat seeds new lines harvested between 1997 to 1999 years were characterized by basic chemical composition and biological activities in relation to agroenvironmental factors such as fertilisation, precipitation and air temperature. Ash content, fat and reducing sugars as well as the level of extractable proteins, amylolytic and inhibitory activities tested against bovine pancreas trypsin and α -amylases of mammalian and insect origin were strongly affected by agro-environmental conditions which prevailed during the new oat lines growth. In three consecutive years of grain harvest the moisture and crude protein were also affected, however less by agro-environmental condition. The oat line CHD 1296 (registered as Hetman variety in 1999) showed quite stabile and high crude protein content over 13%, irrespective of different agro-environmental conditions during three consecutive years of growth.

Key words: oat grains, agro-environmental condition, chemical composition, extractable proteins, biological activities

INTRODUCTION

Among cereals, oat offers a particularly interesting chemical composition and considerable content of dietary fibre, recognized often as a raw material characterized by high nutritive value [Górecka and Stachowiak 2002]. Oat is widely cultivated in temperate regions with a total world production 26.1 mln ton in 2000 [www.stat.gov.pl, Zhou et al. 1998]. This cereal is used by people in three ways: as fodder for animals, as food for human consumption and also as a component to the pharmaceutics and cosmetic industries. Only a very small proportion of oat, as little as 7% of the total crop, is utilized in human nutrition, however in Poland even less, i.e. only about 3% [Owies... 1995]. Nevertheless, human consumption of oat grain increases, and this trend can be

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expected to continue, as well as consumers' demand to increase the production of healthy and nutritious foods. Dolatowski et al. [2002] reported the new possible application of oat grain after hydrothermal treatment in meat products. The nutritional value of oat grain is higher than of other cereals, due to higher content of protein, minerals, dietary fibre and in the case of fat, about two to five times as high as that found in wheat [Kent 1975, Zhou et al. 1998, Górecka and Stachowiak 2002]. Oat also has a better proportion of nonsaturated to saturated fatty acids [Robert et al. 1983, Bartniak and Rothkaehl 1997], as well as higher content of exogenous amino acids, especially lysine [Wu 1983, Owies... 1995]. Basic components of oat are non-starchy polysaccharides and among them beta-glucans. The hypocholesterolemic action of oats flour and oats flakes has been reported earlier, while the β-glucan fraction has been found to be responsible for lowering cholesterol level in blood. However, excessive consumption of oat and its products, especially oat flakes and preparations with high content of dietary fibre, may impoverish the organism of zinc and copper ions [Górecka and Stachowiak 2002]. Oat grains like other cereals contain endogenous proteins being natural inhibitors of enzyme action, as for example, the inhibitors of proteases and amylases [Warchalewski 1983, Andrzejczuk-Hybel 1995, Mikola and Mikkonen 1999]. These inhibitors were extensively studied in other cereals and seeds of pigeon pea [Warchalewski 1987, Warchalewski et al. 1989, Ambekar et al. 1996, Warchalewski et al. 1998, Piasecka-Kwiatkowska 1999, Piasecka-Kwiatkowska and Warchalewski 2000 a, Piasecka-Kwiatkowska and Warchalewski 2000 b] as antinutritional factors and as possible defence components against pests [Warchalewski et al. 2002]. Still there is an insufficient information about native proteinaceus inhibitors, their level and function in oat grain. Mikola and Mikkonen [1999] report the presence of chymotrypsin and trypsin inhibitors in ungerminated oat grain. Probably oat grains have generally lower level of inhibitory activity that was measured in the albumins fraction from other cereals, mainly

Environmental factors such as soil, climate, fertilisation, temperature and rainfall can have significant influence on quantity of crude protein, soluble protein and their different subunits proportion, as well as fat, β-glukan and reducing sugars content. The biological activity of cereals grain like amylolytic activity and inhibitors activity against exogenous α-amylases may have been also influenced by environmental factors [Humphreys et al. 1994, Johansson and Svensson 1998, Piasecka-Kwiatkowska 1999]. In wheat seeds the genetically determined influence of the variety (genotype) is connected with the structure, as well as with the quantity of proteins, whereas the influence of growing conditions is based only on quantitative effect [Wieser and Seilmeier 1998]. In particular, different levels of nitrogen fertilisation are known to cause changes in flour protein quantities and their proportions. The genotype has strong influence on protein concentration in cereals [Welch and Leggett 1997, Gwal et al. 1999, Doehlert and McMullen 2000]. Also fat, ash, β-glucan and non-starchy polysaccharides concentrations are affected by genotype [Doehlert and McMullen 2000, Givens et al. 2000]. Other authors report that the crop yield and the crude protein content increase with increasing nitrogen fertilisation [Jamal et al. 1996, Welch and Leggett 1997, Oscarsson et al. 1998, Gwal et al. 1999]. On the other hand, Wieser and Seilmeier [1998] reported that different level of nitrogen fertilisation strongly influences only the quantity of storage proteins but not other endosperm proteins like albumin and globulin. Variety dependent differences demonstrate that the effect of fertilisation is also determined by genetic factors.

The weather conditions like low rainfall and high average temperature come in on the critical growth stages [Isaac and Hrimat 1999]. The grain proteins content was increased by low water stress particularly at anthesis [Jamal et al. 1996]. Generally the growth and development of oat grain is connected with the good water access [Owies... 1995].

The objective of this study was to determine the level of some chemical composition and biological activities in the new oat lines harvested over three consecutive years in relation to selected changes in agro-environmental conditions.

MATERIAL AND METHODS

The studies were carried out on four new lines of oat grain: CHD 1095, CHD 1296, CHD 1396 and CHD 1496, harvested in 1997, 1998 and 1999. In the meantime the line CHD 1296 was registered in Poland as Hetman variety in 1999 and the line CHD 1396 as Deresz variety in 2000. In 1999 oat lines CHD 1095 and in 2000 CHD 1496 were withdrawn from the further breeding trials. The oat line CHD 1496 was good only in the mountainous area. The doses of nitrogen, phosphorus and potassium fertilisation depended on the requirements of soil, oat genotype and expected yield. Grain samples were obtained from the Breeding Station DANKO in Choryń, Poland. The growing conditions of the new oat lines harvested in the years 1997 to 1999 were given in Table 1.

The α -amylase from porcine pancreas and human saliva was obtained from Sigma Chemical Company and bovine pancreas trypsin from Calbiochem AG. The α -amylase from insects sources were own preparations as described earlier by Warchalewski et al. [1989]. The specific activity [units/mg of protein] of used enzymes was as follows: Sitophilus granarius α -amylase – 28.3 UAA/mg protein, Tribolium confusum α -amylase – 12.5 UAA/mg protein, Ephestia kuehniella α -amylase – 3.3 UAA/mg protein, human saliva α -amylase – 713.4 UAA/mg protein, porcine pancreas α -amylase 438.5 UAA/mg protein and bovine pancreas trypsin 19.4x10⁻⁴ UPA/mg protein.

Grain moisture of cereal samples was determined using moisture-air-oven method, drying at 135° C according to AACC Approved Methods – Method 44-19. The crude protein of the grain samples was determined according to Kjeldahl method with the use of the Swedish Tecator apparatus and the calculating index N = 6.25. The ash and fat were determined according to Polish Methods: PN A-74014:1968 (ash) and PN A-7438:1964 (fat).

The extraction procedure for obtaining albumins extracts from grain was exactly the same as elaborated earlier [Warchalewski et al. 1997]. The extractable proteins content was determined using Lowry method [Lowry et al. 1951]. The reducing sugars content in extracts of albumins was determined by the method of Hostettler and Denel [1951].

Endogenous amylolytic activity (total α - and β -amylase activity) was measured by colorimetric Bernfeld method [1955] using 3.5-dinitro-salicylic acid at pH 5.5 and 487 nm as was modified by Warchalewski and Tkachuk [1978]. A unit of amylolytic activity (UAA) is the amount of enzyme necessary to liberate dextrin's or maltose equivalent to 1 μ mol maltose in 1 min at 25°C from 0.9% soluble starch at pH 5.5.

To determine exogenous α -amylase inhibitory activity against α -amylases from grain insects: *Tribolium confusum* Duv. (larvae), *Sitophilus granarius* L. (imago) and *Ephestia kuehniella* Zell. (young larvae), as well as against mammalian α -amylases from: human saliva and porcine pancreas the modified Bernfeld method [1955] was used

Table 1. Growing conditions of the new oat lines harvested in the years 1997-1999 Tabela 1. Warunki glebowo-klimatyczne w latach zbiorów 1997-1999

Year of harvest Rok zbioru	1997	1998	1999
Fertilisation dose, kg/ha Dawka nawożenia, kg/ha			
N	67	43	70
P_2O_5	69	40	30
K_2O	90	60	45
Average precipitation, cm ³ * Średnie opady, cm ³ *			
January – Styczeń	7.0	39.4	44.0
February – Luty	31.9	33.2	48.2
March – Marzec	26.3	49.3	61.5
April – Kwiecień	29.0	34.5	74.5
May – Maj	59.4	16.5	62.3
June – Czerwiec	19.7	163.0	102.0
July – Lipiec	169.5	96.0	39.5
August – Sierpień	180.0	18.0	_
Total - Razem	522.8	449.9	432.0
Average air temperature, °C Średnia temperatura, °C			
March – Marzec	2.9	3.8	2.3
April – Kwiecień	9.7	10.4	9.3
May – Maj	14.5	14.9	12.6
June – Czerwiec	17.5	17.8	15.4
July – Lipiec	17.9	18.2	20.1
August – Sierpień	17.1	17.6	18.2
Average yield, t/ha Średni plon, t/ha	5.65	7.23	7.33

^{*}January to the first ten-days-period of August.

as described by Warchalewski et al. [1989]. The inhibition activity is a drop of amylolytic activity of α -amylases used and was given in UAA.

Antitryptic activity of the protein extracts was determined basing on the method of Namoto and Narahashi [1959] as modified by Warchalewski and Skupin [1973]. The antyproteolytic activity (UPA) has been expressed as the number of proteolytic activity units by which the enzyme activity has been diminished as a result of the inhibitory action of the inhibitor.

All chemical determinations were conducted in duplicate or triplicate. Statistical differences among years and lines were estimated by two-way analysis of variance (ANOVA) test at the 5% level ($\alpha=0.05$) of significance for all parameters evaluated. Whenever ANOVA indicated significant differences, a pairwise comparison of mean by least significant difference (LSD) was carried out. Statistical analyses of data are given at each figure. The same letter means that data are not significantly different at the level $\alpha=0.05$.

^{*}Od stycznia do pierwszej dekady sierpnia.

RESULTS AND DISCUSSION

According to Gasiorowski [Owies... 1995] the oat grain well grows in the areas with abundant rainfall. Nitrogen fertilisation is very desirable as a protein and yield production factors. The phosphatic fertilisers speed the maturating the plant in the case of a good soil and a lot of precipitation. The potassic fertilisers are assimilated faster by the plant than the phosphatic fertilisers. But the potassium plays a minor part in the oat cultivation because this crop has a large ability to take this component from the soil. The fertilisation doses are dependent on the plant demand as well as on the soil type. The doses of fertilisation given in three consecutive years of harvest were various due to different type and requirements of soil. For example in 1997 the soil was faulty wheat complex while in 1998 and 1999 it was a good wheat complex. As can been seen in Table 1, the average yield of crop was different and resultant of different levels of nitrogen fertilisation and an unequal precipitation in three consecutive years. In 1997 the precipitation was extremely low from April to June, the months crucial to oat growing, while in July and August it was extremely high. On the other hand for oat harvested in 1998 the recorded precipitation only in June was 36% of the total rainfall which in consequence increased grain yield. Generally sufficiently high rain precipitation in April to June and much lower in July and August increased the protein and cereal grain yield.

Oat seeds harvested in three consecutive years showed different moisture and ash content (Fig. 1). Statistical analysis of data provided significant differences between oat lines as well as the years of harvest. Grain moisture of oat harvested in 1997 was higher than in two remaining harvest years and well correlated with unequal rain precipitation in months April to August. In 1999, the ash content in all seeds of the investigated oat was the highest and statistically significant with the lowermost moisture of all oat grains. In the same year the highest nitrogen fertilisation was applied and the lowermost level of precipitation in the period of growth was noticed (Table 1). Oomah [1983] reported even the highest content of ash 3.07% in oat grains. The crude protein content in the tested oats seeds was statistically different among lines as well as between years (Fig. 2). Oat lines with the exception of CHD 1095 showed the highest crude protein content in 1998. The lowermost nitrogen fertilisation in this year was recompensed by abundance of rainfall in June – 163 cm³ and higher air temperature in crucial months of growing (Table 1). April to June 1999 was cooler comparing to the same period of 1998 (Table 1). Therefore, lower level of crude protein in 1999, the year of harvest, can be attributed to low average temperature of air, the lowest phosphatic fertiliser applied and unfavourable rainfall disposition in April to June, even with the highest nitrogen fertilisation. On the other hand, nitrogen fertilisation strongly influenced the yield of crop in 1999 (Table 1). Gąsiorowski et al. [1997] reported that average content of crude protein was 11.7% in 2051 of oat grain samples studied. Zhou et al. [1998] pointed out that 10--12% of crude protein is typical in oat grains. The oat line CHD 1296 showed quite stable and high crude protein content over 13%, irrespective of different fertilisation used, air temperature and precipitation during three consecutive years of growth (Fig. 2 and Tab.1). The fat content in tested oat grain was statistically different between oat lines and years of harvest (Fig. 2). In new oat lines, higher fat content in both crops harvested in 1997 and 1998 was well correlated with favourable temperature of air. In these years average temperatures in April to June were higher than in 1999 (Table 1). Average fat content in studied oat new lines was 4.68% and was lower than the mean

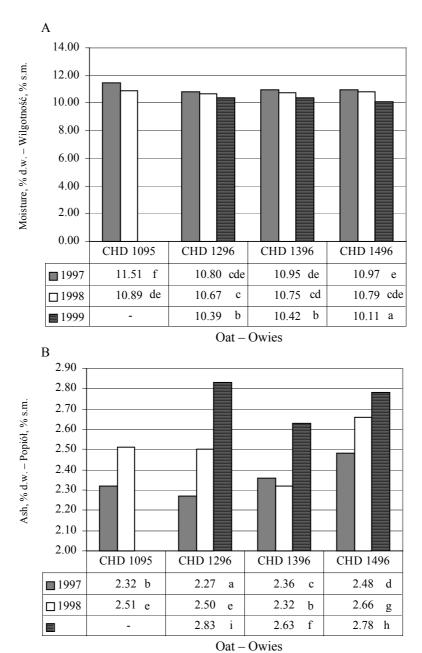


Fig. 1. Moisture and ash content in the new oat lines harvested in three consecutive years, A- moisture, B- ash

Ryc. 1. Wilgotność i zawartość popiołu w ziarnie owsa nowych rodów zebranego w trzech kolejnych latach, A – wilgotność, B – popiół

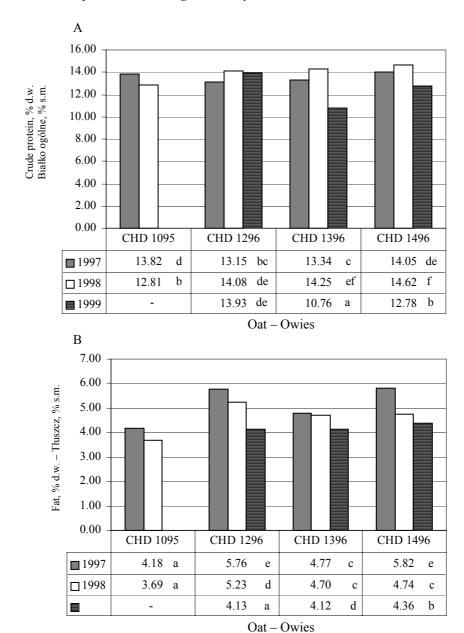


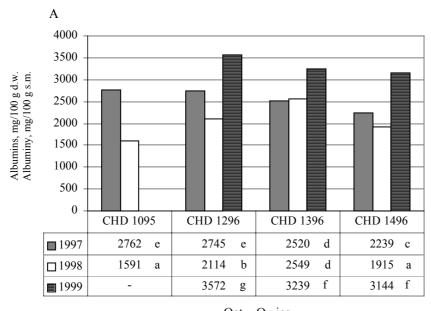
Fig. 2. Crude protein and fat content in the new oat lines harvested in three consecutive years, A- crude protein, B- fat

Ryc. 2. Zawartość białka ogólnego i tłuszczu w ziarnie nowych rodów owsa zebranego w trzech kolejnych latach, A – białko ogólne, B – tłuszcz

value 5.16% given by Gąsiorowski et al. [1997], however higher (3.4%) than was published by Oomah [1983]. According to Gąsiorowski et al. [1997] fat content in oat is genetically dependent to a higher degree than to agro-environmental factors.

Most of papers published on cereals, in particular wheat, indicate that endogenous α -amylase and α -amylase inhibitors were mainly located in albumins protein fraction [Warchalewski and Tkachuk 1978, Warchalewski 1983, Warchalewski et al. 1989, Warchalewski et al. 1998, Piasecka-Kwiatkowska 1999, Piasecka-Kwiatkowska and Warchalewski 2000 a]. Therefore, it was suitable to apply water extraction in order to study these biological activities in oat grain. Water soluble proteins (albumins) had diverse quantitative level in all our oat grain samples studied (Fig. 3). Statistical analysis of data provided the significant differences between oat lines, as well as the year of harvest in the case of albumins fraction and the reducing sugar content (Fig. 3). In 1999 the amount of albumins fraction extracted was the highest in all oat lines, when the higher nitrogen fertilisation was applied and the lowermost rainfall and air temperatures between April to June were recorded (Table 1). The average level of extracted albumins in oats grain was similar to that found in other cereals [Warchalewski et al. 1998]. In addition, the amount of oat albumins was higher than these found in wheat grain, but lower than were found in triticale and rye grain. On the contrary to wheat grain [Wieser and Seilmeir 1998] the level of nitrogen fertilisation of oat strongly influenced the quantity of extracted albumins. Low water stress at anthesis in 1999 increased the level of oat albumins (Fig. 3) similar to wheat proteins reported by Jamal et al. [1996]. On the other hand, high water stress in 1997 significantly increased the level of oat reducing sugars (Fig. 3) which well corresponded with the highest precipitation and grain moisture (Table 1, Fig. 1), favourable conditions to action of endogenous amylases. The level of the reducing sugars found in the investigated oat grains was higher when compared to other cereals grains [Kuśnierz et al. 1999]. Moreover, it was higher than the content of reducing sugars given by Zhou et al. [1998].

All biological activities studied in oat grain lines showed statistically significant differences between individual oat grain samples and between the years of harvest (Fig. 4-7). The level of extracted albumins from grain of the new oat lines well correspond to the total amylolytic activity determined (Fig. 3 and 4). The total α - and β -amylase activity was greatly lower when compared with the same activity found in other cereals grains [Warchalewski et al. 1998]. Figure 5 shows the inhibition activity against α -amylase from insect sources. The degree of inhibition was different to individual oat grain samples and years of crop. The inhibitors determined in the investigated oats grain were much more active against Tribolium confusum α -amylase than against α -amylase of Sitophilus granarius and was similar to wheat varieties studied by Warchalewski et al. [1989]. Furthermore, the inhibition activity against α-amylase of Ephestia kuehniella Zell. was not detected in the case of any oat grain and year of harvest. Two oat lines CHD 1296 and CHD 1496 harvested in 1999, had the highest inhibitory activities against α-amylases of both insects (Fig. 5) which were in line with the highest extracted protein (Fig. 3) and nitrogen fertilisation used (Table 1). Also the highest level of inhibition activity was found in extractable proteins of all oat grain samples harvested in 1999 in the case of inhibitory activity against α -amylase from human saliva (Fig. 6). The inhibition activity towards porcine pancreas α -amylase was different when compared to individual oat grain sample and lower than in the case of human saliva α -amylase.



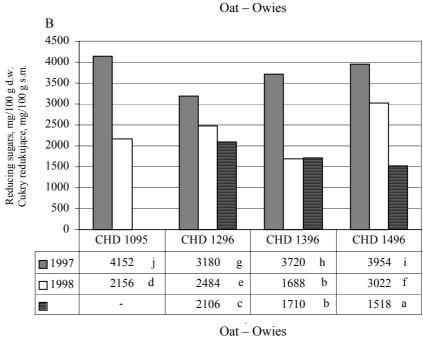
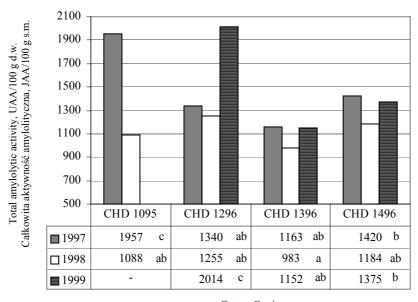


Fig. 3. Quantities of extracted albumins and reducing sugars in the new oat lines harvested in three consecutive years, A – albumins, B – reducing sugars Ryc. 3. Ilość białka wyekstrahowanego i zawartość cukrów redukujących w ziarnie owsa nowych rodów zebranego w trzech kolejnych latach, A – abluminy, B – cukry redukujące

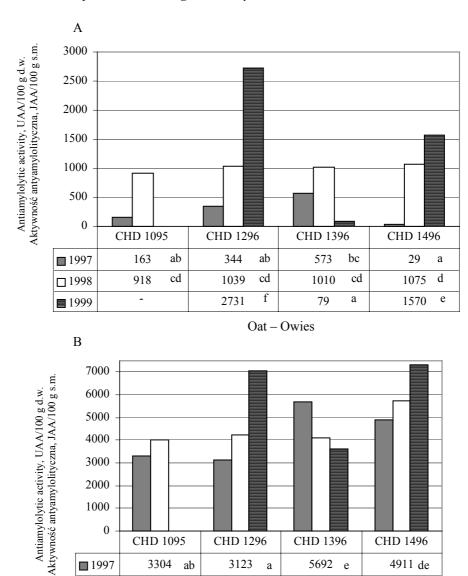


Oat - Owies

Fig. 4. Total (α - and β -) amylolytic activity determined in extractable protein from grain of the new oat lines harvested in three consecutive years

Ryc. 4. Całkowita aktywność (α- i β-) amylolityczna oznaczona w wyekstrahowanym białku z ziarna owsa nowych rodów zebranego w trzech kolejnych latach

Moreover, the inhibition activity in the case of porcine pancreas α -amylase (Fig. 6) and trypsin (Fig. 7) was not detected in all oat grains harvested in 1998. Perhaps the extremely low level of applied nitrogen fertilisation in 1998 (Table 1) could restrict the synthesis of this part of albumins which was responsible for inhibitory activities against porcine pancreas α -amylase and trypsin. In this year the lowest level of extracted albumins from grain of all studied oat lines was recorded (Fig. 3). It should be mentioned that all biological activities determined in grain of the new oat lines (Fig. 4-7) irrespective of the years of harvest were considerably lower than published earlier in the case of wheat, rye and triticale [Warchalewski et al. 1989, Warchalewski et al. 1998, Piasecka-Kwiatkowska 1999]. Therefore, much lower inhibitory activities towards mammalian αamylases and trypsin (Fig. 6 and 7) in the new oat lines grain should not cause any nutritional problems. This suggestion was supported by Mikola and Mikkonen [1999]. They found at least two types of both trypsin and chymotrypsin inhibitors in oats. Most oat chymotrypsin and trypsin inhibitors are heat labile and sensitive at low pH. These inhibitors had low activity that is why their inactivation at low pH, like in human stomach and high temperature can be easily achieved. Moreover, oat grains for human use are usually processed including heat treatment to prevent spoiling. Oat proteins with activity against insects α -amylases may have some influence on reduction of insects populations like in the case of other cereals [Baker et al. 1991, Zhang et al. 1997, Warchalewski et al. 2002]. Although, whether inhibitors of insects α-amylase can play any function as defence proteins towards T.confusum and S.granarius in oat seeds is still an open question and remains for further investigation.



Oat - Owies

a

CHD 1396

5692 e

4086 bcd

3613 abc

CHD 1496

4911 de

5727 e

7319 f

Fig. 5. Inhibition activity of oat albumins against α -amylases from insect sources, A – inhibition activity against Sitophilus granarius L. α-amylase, B - inhibition activity against Tribolium confusum Duv. α-amylase

CHD 1296

3123

7053

4222 cd

Ryc. 5. Aktywność hamująca działanie owadzich α -amylaz oznaczona w albuminach owsa, A – aktywność inhibitorowa wobec α-amylazy Sitophilus granarius L., B – aktywność inhibitorowa wobec α -amylazy Tribolium confusum Duv.

0

■ 1997

□ 1998

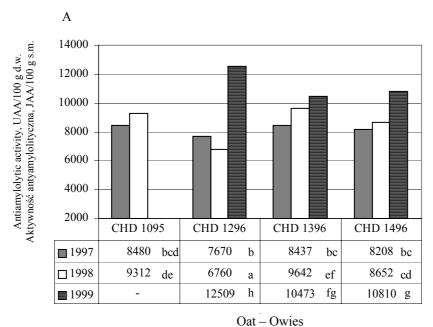
CHD 1095

3304

4000

ab

bc



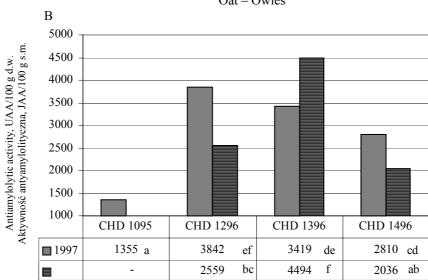


Fig. 6. Inhibition activity of oat albumins against α -amylases from mammalian sources, A – inhibition activity against human saliva α -amylase, B – inhibition activity against porcine pancreas α -amylase

Oat - Owies

Ryc. 6. Aktywność hamująca działanie α -amylaz ssaków oznaczona w albuminach owsa, A – aktywność inhibitorowa wobec α -amylazy śliny ludzkiej, B – aktywność inhibitorowa wobec α -amylazy trzustki wieprzowej

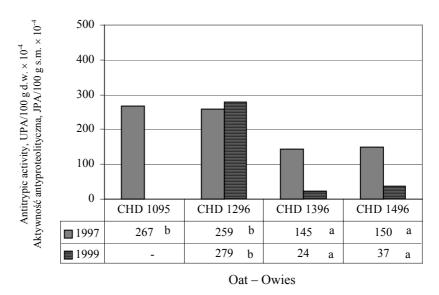


Fig. 7. Inhibition activity against bovine pancreas trypsin determined in extractable protein from grain of the new oat lines harvested in three consecutive years

Ryc. 7. Aktywność antyproteolityczna wobec trypsyny wołowej oznaczona w białku wyekstrahowanym z ziarna owsa nowych rodów zebranego w trzech kolejnych latach

CONCLUSIONS REMARKS

The highest ash content statistically significant was determined in oat grains harvested in 1999, when the lowermost rainfall was recorded and the highest nitrogen fertilisation used. Oat seeds harvested in 1997 showed the highest value of reducing sugars which was statistically significant and well corresponded to the highest precipitation, grain moisture and high endogenous amylolytic activity. The new oat line CHD 1296 (registered as Hetman variety in 1999) showed quite stable and high crude protein content of over 13%, irrespective of different agro-environmental conditions during three consecutive years of growth. Fat content in oat grains harvested in 1997 and 1998 was well correlated with higher air temperature in April to June. High nitrogen fertilisation caused a significant increase of water extractable proteins content and amylolytic activity from oat seeds. Extractable proteins of oat grain studied showed the presence of inhibition activities against α-amylases from T.confusum, S. granarius, human saliva, porcine pancreas and bovine pancreas trypsin. The level of these inhibition activities was strongly dependent on changes in agro-environmental conditions during oat seeds growth. No inhibition activity against E.kuehniella α-amylase has been detected in the studied oat lines.

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SKŁAD CHEMICZNY I AKTYWNOŚCI BIOLOGICZNE ZIARNA OWSA NOWYCH RODÓW W ODNIESIENIU DO WARUNKÓW GLEBOWO-KLIMATYCZNYCH W LATACH 1997-1999

Streszczenie. Ziarno owsa czterech nowych rodów, zebrane w latach 1997-1999 scharakteryzowano pod względem podstawowego składu chemicznego i aktywności biologicznych w odniesieniu do warunków glebowo-klimatycznych, takich jak nawożenie, opady atmosferyczne i średnie miesięczne temperatury. Zawartość popiołu, tłuszczu, cukrów redukujących, ilość białek wyekstrahowanych, aktywność amylolityczna oraz inhibitorowa wobec trypsyny wołowej, jak też α-amylaz ssaków i owadów ulegała zmianom pod wpływem zmiennych warunków glebowo-klimatycznych panujących w różnych latach zbiorów. Wilgotność ziarna i zawartość białka ogólnego w badanych rodach owsa podlegała mniejszej zmienności pod wpływem warunków glebowo-klimatycznych. Ród owsa CHD 1296 (zarejestrowany w 1999 roku jako odmiana 'Hetman') odznaczał się stabilną i wysoką zawartością białka, powyżej 13%, niezależnie od różnych warunków glebowo-klimatycznych panujących w trzech kolejnych latach zbiorów.

Slowa kluczowe: ziarno owsa, warunki glebowo-klimatyczne, skład chemiczny, białka rozpuszczalne, aktywności biologiczne

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