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The influence of γ-irradiation on some biological activities and electrophoresis patterns of wheat grain albumin fraction

J. Gralik *, J.R. Warchalewski

August Cieszkowski Agricultural University of Poznań, Department of Food Biochemistry and Analysis, Mazowiecka 48, 60-623 Poznań, Poland

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Abstract

Water-soluble proteins were extracted from irradiated wheat grain for the purpose of assaying biological activities, reducing sugars content and SDS–PAGE electrophoresis. The differences between samples after γ-irradiation were tested by analysis of variance at the level of significance ($P \leqslant 0.05$). Ionising radiation (0.05–10 kGy) caused an increase in the activity of endogenous amylases, statistically significant at doses of 5 and 10 kGy ($P \leqslant 0.05$), which was well correlated with the highest extractable protein content. γ-Irradiation of wheat grain at a dose of 0.05 kGy caused an increase of inhibition activity against *Sitophilus granarius* L. α-Amylase, whereas there was a decrease at 10 kGy ($P \leqslant 0.05$). On the other hand, grain irradiated by 0.5 and 1 kGy doses showed a significant increase in inhibition activity against α-amylase of *Tribolium confusum* Duv. ($P \leqslant 0.05$) while at the remaining doses the inhibition activity was on the same level as the control grain. Decrease of *Ephesitia kuehniella* Zell. α-amylase was observed only at 5 kGy radiation dose ($P \leqslant 0.05$). At the remaining doses this activity was comparable to non-irradiated grain.

The highest increase of antiamylolytic activity against human saliva α-amylase was noted in albumin proteins of wheat grain treated by γ-radiation doses >0.5 kGy while there were decreases at radiation doses <0.5 kGy ($P \leqslant 0.05$). The increase of inhibition activity against hog pancreas α-amylase was noted at radiation doses 0.1 kGy ($P \leqslant 0.05$). γ-Radiation doses of 0.05, 0.5 and 1 kGy caused an increase of the antitryptic activity while 10 kGy decreased this activity ($P \leqslant 0.05$). SDS–PAGE electrophoresis patterns of albumin sample at 0.1 kGy showed 15 proteins while in the control sample only 11 bands were found. Among studied wheat albumins, the lower protein bands 5 and 8 were detected in wheat grain samples irradiated by 10 and 0.5 kGy, respectively. The differences in inhibitor activities and electrophoretic protein bands of wheat albumin from γ-irradiated wheat grain give additional evidence that these proteinaceous inhibitors against enzymes studied are different in nature.

Keywords: γ-Irradiation of wheat grain; Reducing sugars content; Endogenous amylase activity; Inhibitors of exogenous α-amylases and trypsin; Albumin SDS–PAGE electrophoresis

1. Introduction

γ-Radiation has been used to inhibit sprouting in potatoes and garlic bulbs, to reduce cooking time of legumes, to reduce toxic constituents such as antithiamin in tuna, to reduce flatulence caused by oligosaccharides in green gram, to inactivate inhibitors of chymotrypsin, and to reduce the number or the activity of viable microorganisms in peanuts, barley malt and chickens (Tai-Sun Shin & Godber, 1996).

According to Brader et al., 2002 post harvest food losses are estimated in the range from 9% in the United States up to 50% in some other parts of the world. Much of the loss results from the invasion of the grain mass by mold, insects and rodent pests. In addition, insect infested grains should not be consumed as they may cause serious health hazards in human beings (Samuels & Modgil, 1999). Therefore, the control of insect infestation in stored wheat grain by irradiation has been extensively

* Corresponding author. Fax: +48 61 848 73 52.
E-mail address: jgralik@wp.pl (J. Gralik).

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investigated (Aldryhim & Adam, 1999; Delincee, 1998; Donahaye, 2000). Irradiation is an available alternative to chemical fumigation for significant reduction of grain damage by insect pests (Warchalewski, Gralik, & Nawrot, 2000). γ-Radiation can be used to destroy pests and microorganisms in either bulk or packaged food. Lower doses of γ-radiation up to 0.5 kGy, which are safe from a technological point of view (Błaszczak, Gralik, Klockiewicz-Kamińska, Fornal, & Warchalewski, 2002; Dolińska, Warchalewski, Gralik, & Jankowski, 2004; Klockiewicz-Kamińska, Warchalewski, & Gralik, 2000), do not cause immediate death of adult pests, but can prevent an increase in population by lethal effects on immature stages, sterilisation of adults and reduction in adult longevity (Aldryhim & Adam, 1999; Rao, Gholap, Adhikari, & Nair, 2000). It can also reduce spoilage of foods, prevent contamination by pathogens, and prolong shelf life. There is contradictory information concerning the influence of γ-irradiation on food amino acids composition. Some researchers have not found any difference in amino acids composition of food after γ-irradiation, whereas others found that total protein content was unchanged, however, amino acids composition altered (Glidewell, Deighton, Goodman, & Hillman, 1993). γ-Rays have short wavelengths and are capable of hydrolysing chemical bonds, thereby cleaving molecules into small fragments that may be either electrically charged ions or uncharged free radicals. The degree of cleavage is largely proportional to dose, which may vary considerably depending upon the treatment objective (Bhatty & MacGregor, 1988).

When applying γ-radiation in order to reduce grain damage by insect pests or moulds one should always consider how this treatment will affect wheat grain properties. That is why research on induced changes on wheat grain caused by γ-radiation remains an important and interesting challenge. It has been proved so far that γ-irradiation can change some of the biological, nutritional and technological properties (Błaszczak et al., 2002). Preliminary studies (Prądzyńska & Warchalewski, 1999a, 1999b; Warchalewski, Prądzyńska, Gralik, & Nawrot, 2000) have proved also that changes observed in irradiated wheat grain can change grain susceptibility to the granary weevil (*Sitophilus granarius* Zell.), the confused flour beetle (*T. confusum* Duv.) and Mediterranean flour moth (*Ephesitia kuehniella* Zell.). In addition, γ-irradiation between doses 1 and 10 kGy was found to be more effective for microflora reduction (Gralik, Trojanowska, & Warchalewski, 1999).

Water extractable proteins from wheat grain, namely albumins, have a number of important biological activities such as endogenous amylases, inhibitors of endogenous and exogenous α-amylase and trypsin (Silano, 1987, Chapter 6; Warchalewski, 1983, 1987). These activities have potential physiological, nutritional, toxicological and technological significance (Warchalewski, Madaj, & Skupin, 1989a) and are important when considering resistance of the grain to stored insect pests, which can cause secondary infestation from γ-irradiated wheat grain (Warchalewski, Prądzyńska, et al., 2000).

The purpose of this study was to investigate possible changes in biological activities, reducing sugars content and electrophoretic patterns of albumin fractions, extracted from irradiated wheat grain which can possibly affect grain properties.

2. Experimental

2.1. Wheat grain

Winter wheat Begra variety used for this study was obtained from Plant Breeding Station DANKO in Choryń. Prior to analysis irradiated wheat grain samples were stored in tightly closed containers at 18 °C. The grain moisture was at the mean level of 13.37%, ranging from 13.21% to 13.56%. Total protein content was at the mean level of 14.82% d wt. ranging from 14.71% to 14.96%.

2.2. γ-Irradiation process

γ-Radiation process was conducted in a RChM-γ-20 apparatus, packed with ^{60}Co source (Russia). Wheat grain (5000 g) were divided into five portions. Each portion of 1000 g was irradiated in an aluminium vessel with γ-rays at the following doses: 0.05, 0.1, 0.5, 1, 5 and 10 kGy. The range of damage depends on the dose applied. Doses over 1 kGy were used mainly to monitor changes under extreme conditions of irradiation doses suitable for reduction of microflora. It should be mentioned that doses up to 10 kGy are officially permitted by WHO/FAO in food irradiation. The strength of the radiation dose rate was determined by Fricke's dosimeter and amounted 0.5 Gy/s. Since the after-effect can be observed by liberation of free radicals (Warchalewski, 1983) in this study all analysis were carried out three months after γ-irradiation process.

2.3. Extraction of wheat albumins

Wheat grain samples were ground into whole flour using a laboratory mill. The extracts of albumin proteins used for estimation of biological activities, reducing sugar content and electrophoresis were obtained by means of three step water extraction according to Warchalewski, Piasecka-Kwiatkowska, and Madaj, 1997. Water-soluble proteins were extracted from 3 g dry weight flour in three separate runs at 18 °C by shaking 1 h with 30 ml of distilled water and then centrifuged for 10 min at 20,000*g*. After centrifugation, collected supernatants

were combined and divided into small portions of approximately 5 ml and kept frozen prior to analysis.

2.4. Protein determination in extracts

The extractable proteins content was determined at 750 nm using the Lowry, Rosenbrough, Farr, and Randall (1951) method.

2.5. Assay of reducing sugars content

Reducing sugars content in water extracts was determined according to Hostettler and Denel (1951) at $\lambda = 530$ nm.

2.6. Assay of endogenous amylolitic activity (α- and β-amylase) and inhibitory activities against exogenous α-amylases

Endogenous amylolitic activity (total α- and β-amylase activity) and inhibitory activities against α-amylases from grain insects: *E. kuehniella* Zell. (young larvae), *S. granarius* L. (imago) and *T. confusum* Duv. (larvae) were determined according to the colorimetric Bernfeld method (1955) using 3,5-dinitro-salicylic acid at pH 5.5 and 487 nm as was modified by Warchalewski (1987) and Warchalewski and Tkachuk (1978). Insects' α-amylases were prepared as described earlier (Warchalewski et al., 1989a). The specific activities of insect α-amylases were as following: *S. granarius* L. 25.60 UAA/mg proteins, *T. confusum* Duv. 16.80 UAA/mg proteins, *E. kuehniella* Zell. 0.61 UAA/mg proteins. The measurements of inhibition activity against α-amylase from mammalian sources (human saliva and hog pancreas – Sigma Chemical Co.) were done as described earlier at pH 6.8 using 0.143 M veronal buffer (Warchalewski et al., 1989a). The specific amylolitic activity of human saliva α-amylase was 240.56 UAA/mg proteins and in the case of hog pancreas α-amylase 674.95 UAA/mg proteins.

Under the conditions of the assays one unit of amylolytic activity was defined as the amount of enzyme necessary to liberate dextrin or maltose equivalent to 1 μmol maltose in 1 min at 25 °C from 0.9% soluble starch at pH 5.5.

$$\begin{aligned}\text{Inhibitor activity} = {} & (\text{amylase activity of enzyme} \\ & + \text{endogenous amylase activity of} \\ & \quad \text{inhibitor solution}) \\ & - (\text{remaining amylase activity of} \\ & \quad \text{inhibitor solution}).\end{aligned}$$

2.7. Assay of antitryptic activity

Antitryptic activity was determined against trypsin from porcine pancreas (Sigma Chemical Co.), based on the method of Nomoto and Narahashi (1959), as modified by Warchalewski and Skupin (1973). Specific activity of trypsin form porcine pancreas was 128.82×10^{-4} UAP/mg proteins.

2.8. Electrophoresis SDS–PAGE of wheat albumin

Electrophoresis on 12.5% polyacrylamide gel in the presence of SDS was performed according toMądrzak (1990). In the case of each sample, 10μg of protein was loaded on the gel. Electrophoresis was performed by means of Kucharczyk Minipol Electrophoresis System with a gel 1 mm thick, 10 cm wide and 7 cm long. Molecular masses of the proteins were estimated with the use of Wide Molecular Weight Range Sigma Marker which consists of rabbit muscle myosin (205,000 Da), *E. coli* β-galactosidase (116,000 Da), rabbit muscle phosphorylase b (97,000 Da), rabbit muscle fructose-6-phosphate kinase (84,000 Da), bovine serum albumin (66,000 Da), bovine liver glutamic dehydrogenase (55,000 Da), chicken egg ovalbumin (45,000 Da), rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (36,000 Da), bovine erythrocytes carbonic anhydrase (29,000 Da), bovine pancreas trypsinogen (24,000 Da), soybean trypsin inhibitor (20,000 Da), bovine milk α-lactalbumin (14,200 Da),bovine lung aprotinin (6500 Da). The electrophoretic patterns of wheat albumins were analysed by the computer software GEL SCAN v. 1.45 elaborated by Kucharczyk Electrophoresis Techniques.

2.9. Statistical analysis

The extractable protein content, reducing sugars and all results of biological activities for each replicate were done in triplicate. The differences between samples were verified by analysis of variance. The accepted level of significance was set at $P \leqslant 0.05$. Where it was possible, regression equations and coefficients of determination were calculated also.

3. Results and discussion

Virtually biologically active wheat proteins were found in soluble proteins. The soluble proteins are therefore composed of what is known classically as albumins and globulins, together with glycoproteins, nucleoproteins and many of the lipid–protein complexes found in wheat flour (Kasarda, Nimmo, & Koler, 1971, Chapter 6). Results of biological activities found in the albumin fraction are presented in Table 1. Since variation in moisture content (average 13.37%) was not statistically significant in all analysed samples, γ-irradiation of wheat grain was not affected by the differences in grain moisture.

Table 1
The influence of wheat grain γ-irradiation on albumin chemical and biological properties

Radiation dose (kGy)	Reducing sugar content (mg/100 g d wt.)	Inhibition activity against α-amylase from					Inhibition activity against bovine pancreas typsin (UPA × 10^4/100 gd wt.)
		Sitophilus granarius L. (UIA/100 g d wt.)	*Tribolium confusum*. Duv. (UIA/100 g d wt.)	*Ephesitia kuehniella* Zell. (UIA/100 g d wt.)	Human saliva (UIA/100 g d wt.)	Hog pancreas (UIA/100 g d wt.)	
0	1023a	55,205a	52,286a	11,325a	229,423a	133,092a	9571a
0.05	209b	90,175b	82,791a	10,208a	183,051b	143,555a	14,466b
0.1	2049b	52,538ac	80,717a	12,287a	18,3051b	108,962a	10,128a
0.5	2027b	60,972a	102,061b	13,357a	208,754a	219,390b	143,45b
1	2125c	51,951ac	129,053b	7178a	280,854c	258,764b	13,602b
5	2105c	55,095a	77,666a	3954b	326,755d	232,998b	9677a
10	2262d	44,333c	80,956a	8300a	255,687e	172,962c	3120c

Results with different letters are statistically significant ($P \leqslant 0.05$).

The mathematical model of the influence of γ-ionising radiation on soluble protein content is presented in Fig. 1. Statistical analysis of results shown in Fig. 1 proved statistically significant differences among the extractable protein contents of irradiated samples starting from the dose 0.5 kGy ($\alpha = 0.05$). Within the increasing radiation doses an increase of extractable protein was observed. The increase of extractable protein shown in Fig. 1 is probably due to some changes caused by γ-rays which are capable of breaking covalent bonds thereby cleaving molecules into small fragments as was suggested by Bhatty and MacGregor (1988). Increasing ionising radiation, especially in the range of doses 1–10 kGy, caused some visual changes to the microstructure of wheat kernel endosperm observed by scanning electron microscopy and light microscopy (Błaszczak et al., 2002). As stated earlier (Błaszczak et al., 2002) γ-rays with increasing radiation doses promoted gel-like properties of starch granules and in consequence could facilitate separation of the protein layer surrounding starch granules and in turn increase the extractability of proteins. On the other hand, the breakdown of gluten proteins should be excluded since earlier, under similar conditions, the amount of wet gluten was not affected by γ-rays (Klockiewicz-Kamińska et al., 2000).

A statistically significant increase in reducing sugar content over 100% was noted in all irradiated grain samples (Table 1). We believe that this dramatic increase in reducing sugars was mainly due to some changes in starch granules caused by γ-rays. However, it should be also considered that part of the reducing sugars may have come from other sources such as arabinoxylans. γ-Irradiated wheat grain show a gradual decrease in falling number values and gelatinisation enthalpy, however, which has been shown to be statistically significant at 5 and 10 kGy doses (Dolińska et al., 2004). The increase in reducing sugar supports some changes in the grain starch granules which have been reported earlier (Błaszczak et al., 2002), where ionising radiation caused visible changes in the microstructure of the kernel starchy-endosperm observed at 1 kGy and to a greater extend at 10 kGy dose of radiation examined by SEM and LM. According to Sokhey and Hanna (1993) low radiation doses up to 3 kGy are not visible in the starch granules microscopic appearance, but changes in the granule structure are proved by an increase in the Farrand Equivalent Units, as well as in water binding capacity values at the discussed range of radiation. Also MacArthur and D'Appolonia (1983) believed that the drop in the falling number as a result of γ-rays is due to changes in starch properties rather than in α-amylase activity. However, the statistically significant increase of endogenous amylolitic activity shown in Fig. 2 supports some contribution of amylolitic enzymes as observed by MacArthur and D'Appolonia (1984) in changes of

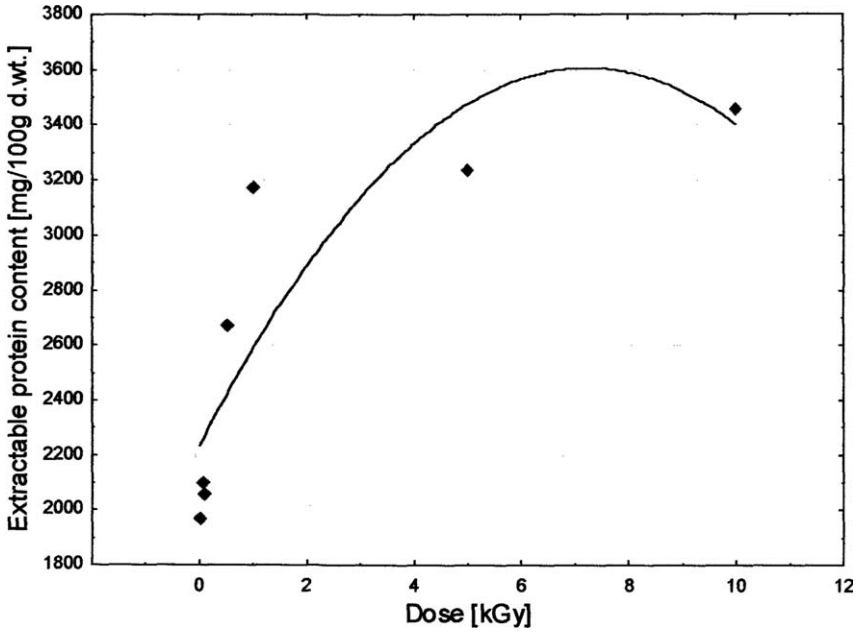

Fig. 1. Extractable protein content of γ-irradiated wheat grain described by the polynomial equation: $y = 2235.8606 + 379.624 \times x - 26.3005 \times x^2$, $R^2 = 0.85$.

starch properties like the reduction of swelling power and the increase in starch solubility of irradiated wheat grain. Farag Zaied, Abdel-Hamid, and Attia (1996) reported a substantial increase in the reducing sugar content, which was accompanied by no changes in the content of starch when grain samples were irradiated up to 8 kGy dose. Therefore, the over 100% increase of reducing sugar content within the radiation doses used (Table 1) may be linked to the increase in starch solubility, making them more susceptible for endogenous amylolitic hydrolysis as well as increasing the extractability of liberated sugars from glycoprotein complexes as suggested by Farag Zaied et al. (1996). Marathe, Machaiah, Rao, Pednekar, and Rao (2002) also noted a distinct increase in reducing sugar content over 100% in whole wheat flour that had been γ-irradiated by 1 kGy dose. Ionising radiation caused an increase of the activity of endogenous amylases (Fig. 2) which was statistically significant from a 5 kGy dose ($P \leqslant 0.05$). Earlier Warchalewski and Klockiewicz-Kamińska (1989)

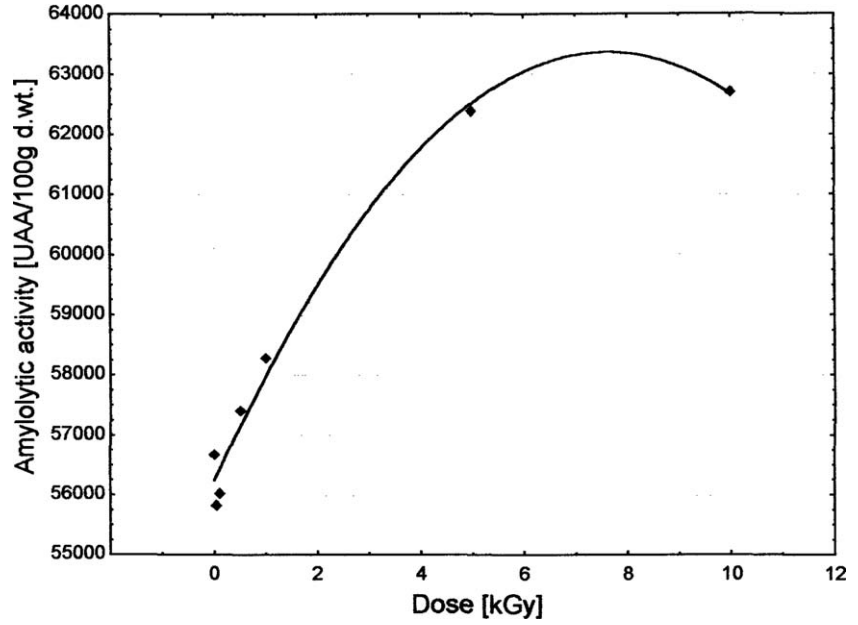

Fig. 2. Amytolitic activity (α- and β-amylase) of gamma irradiated wheat grain described by the polynomial equation: $y = 56231.8769 + 1873.1802 \times x\text{-}122.9469 \times x^2$, $R^2 = 0.89$.

reported that γ-radiation doses of 0.5 kGy caused an increase of amylolitic activity by 5%, in grain of winter wheat beta variety. The increase in amylolitic activity at radiation doses 1, 5 and 10 kGy (Fig. 2) was well correlated with the highest extractable protein content (Fig. 1) as well as the drop in falling number values and observed changes in protein bodies adhering to starch granules giving an impression of fragility (Błaszczak et al., 2002). Hudson (1989) suggests that ionising radiation brings about the destruction of cell structure and in turn releases enzymes outside of the cells causing indirectly an increase in their activity as in case of amylolitic activity shown in Fig. 2.

Cereals contain proteins which are enzyme inhibitors and among the most wide spread of such compounds are inhibitors of hydrolytic enzymes as, for example, the inhibitors of α-amylases (Deponte, Parlamenti, Petrucci, Silano, & Tomasi, 1976; Silano, 1987; Warchalewski, 1983, 1987; Vittozi & Silano, 1976). These inhibitors are water-soluble (Deponte et al., 1976; Warchalewski et al., 1997) and play essential nutritional (Choudhury, Maeda, Murayama, & Dimagno, 1996) and technological roles (Zawistowska, Langstaff, & Bushuk, 1988) as well as increasing natural resistance against stored insect pests (Silano, 1987; Warchalewski & Nawrot, 1993; Warchalewski, Gralik, Winiecki, Nawrot, & Piasecka-Kwiatkowska, 2002). Now it is well established that wheat albumins that make up about 2/3 of the whole albumin of the wheat kernel contain three heterogenous families of inhibitors with molecular masses of 60,000, 24,000, 12,000 Da which may represent as much as 1% of wheat flour (Deponte et al., 1976; Silano, 1987). In the presence of sodium dodecyl sulphate (SDS) as in the case of SDS–PAGE electrophoresis used in this study, all isoinhibitors with molecular masses 60,000 and 24,000 Da were dissociated reversibly to monomeric subunits with molecular masses close to 12,000 Da as reported earlier by Silano (1987). The majority of purified inhibitors from cereals were not determined against their native α-amylase (Warchalewski, 1987). Only the inhibitors WASI and A/T-WI from wheat and the two inhibitors from barley showed inhibitory activity towards their endogenous α-amylases. The bifunctional endogenous α-amylase and trypsin inhibitor coded A/T-WI had molecular mass 23,233 Da which in the presence of SDS also dissociated into two monomeric subunits with molecular masses of 11,500 and 10,800 Da, respectively (Warchalewski, 1987). Therefore, in SDS–PAGE electrophoresis conditions used in this paper we can expect only monomeric isoinhibitors of α-amylases which can be probably located in the bands numbered 14 and 15 (Table 2).

In earlier studies (Warchalewski, 1983), all wheat grain samples irradiated by doses of 0.01–7 kGy caused disappearance of inhibitory activities against wheat native α-amylase and amylases from *Bacillus subtilis* and *Aspergillum oryzae*. Only inhibitory activity against hog pancreas α-amylase was not destroyed by γ-rays. It is possible that some increase in amylolitical activity (combined α- and β-amylase activities) which can be seen in Fig. 2 can be partially due to inhibitor inactivation of their native α-amylase caused by γ-irradiation. This suggestion was supported additionally by the decrease in the falling number values with increasing γ-radiation doses used (Błaszczak et al., 2002). γ-Irradiation caused the increase of inhibition activity of *S. granarius* L. α-Amylase which was statistically significant only at the dose 0.05 kGy and the decrease at 10 kGy ($P \leqslant 0.05$). Extractable protein obtained from γ-irradiated wheat grain at doses of 0.5 and 1 kGy caused an increase in inhibition of *T. confusum* Duv. α-amylase activity ($P \leqslant 0.05$) while 5 kGy dose caused the decrease of this activity against

Table 2
Molecular masses (Da) of wheat albumins obtained from γ-irradiated grain after separation by SDS–PAGE electrophoresis

Bands number	Dose (kGy)						
	0	0.05	0.1	0.5	1	5	10
1	–	147,780	147,780	–	–	–	–
2	131,547		131,546	–	–	–	–
3	–	–	99,162	–	105,980	–	–
4	–	–	87,538	–	–	–	–
5	–	–	–	–	67095	–	–
6	62,259	60,726	60,726	58,740	61,743	61,232	62,259
7	50,577	48,923	49,332	49,744	49,331	48,518	–
8	44,649	44,279	43,549	–	44,279	44,279	–
9	40,075	39,089	41,776	39,744	39,089	39,089	–
10	28,980	28,740	28,740	28,980	28,740	28,740	28,980
11	25,583	25,161	25,161	25,161	25,371	25,371	25,161
12	20,783	19,125	20,783	–	20,783	20,957	23,155
13	17,895	17,025	17,309	17,310	17,310	16,884	–
14	14,062	13,716	13,602	13,946	13,946	14,062	–
15	–	11,050	11,329	–	11,712	11,712	–
16	7415	7539	7665	6600	7602	7665	8756

E. kuehniella Zell. α-amylase ($P \leqslant 0.05$) which can be seen in Table 1. The variations in these inhibitors activities against insects' α-amylases, as a response to applied γ-rays, give evidence that the inhibitors found in extractable protein are different. In general, cereal α-amylase inhibitors may play a protective role against insects although it appears that some insects are able to either detoxify these compounds or increase production of their own α-amylase as in the case of *T. confusum* Duv. (Gatehouse, Fenton, Jepson, & Pavey, 1986). Our results published in this paper referring to our earlier papers (Warchalewski, Gralik, et al., 2000; Warchalewski, Gralik, et al., 2002) support the suggestion of Gatehouse et al. (1986) that selecting wheat varieties for high α-amylase inhibitory activity may not be a very reliable criterion when selecting for natural insect resistance. On the other hand, development time expressed in days in the case of *S. granarius* L. were approximately 19 and 21 days longer on grain samples irradiated with 0.5 and 5 kGy doses as reported earlier (Warchalewski, Gralik, et al., 2000). A similar situation was observed also in the case of *T. confusum* Duv. where 7 days extension of development time was noted when this insects infested 0.1 kGy γ-irradiated grain samples (Warchalewski, Gralik, et al., 2000). Probably the other properties of these γ-irradiated grain contributed to the beneficial extension of development time, since changes in the antiamylolitic activity were not statistically significant at 0.1, 0.5 and 5 kGy (Table 1). Similarly to our earlier papers (Warchalewski et al., 1989a; Warchalewski & Nawrot, 1993; Warchalewski, Gralik, et al., 2002) γ-irradiated wheat grain samples have lower inhibitory activity against α-amylase of *E. kuehniella* Zell compared to *S. granarius* L. and *T. confusum* Duv. (Table 1). The enzymology of the insects' digestive enzymes in relation to initial stages of digestion of large food polymers like starch and proteins reflects the biochemical adaptation of these post harvest insects to their preferred foods (Baker, 1986). α-Amylases of *E. kuehniella* Zell. probably play a minor role since the ratio of α-amylase/proteinase digestive enzymes was 7.9, whereas in *S. granarius* L. it was more than 822 as reported by Baker (1986). No relation was founded between statistically significant changes in antitryptic activity (Table 1) and development time of *E. kuehniella* Zell. published earlier (Warchalewski, Prądzyńska, et al., 2000). Recently, it was stated that even though the inhibitors in wheat-based diets are highly active against the digestive enzymes of these insects they have only limited influence on the development parameters in general causing weakness in the larval condition (Warchalewski, Gralik, et al., 2002). Substantial increases in inhibition activities against α-amylases from mammalian sources (Table 1) should be considered as a disadvantage of irradiated wheat grain from the nutritional point of view. Generally, an increase of biological activities, studied in irradiated wheat grain are mainly due to an increase in extractability of these proteins (Fig. 1).

The SDS–PAGE electrophoresis of albumin proteins extracted from gamma irradiated wheat grain samples after separation were analysed by GEL SCAN in order to draw the densytograms shown in Fig. 3 and estimated molecular masses of protein bands tabulated in Table 2. The grain samples treated at 0.1 kGy have been shown to have more protein bands (15 bands) while in the control samples (0 kGy) only 11 protein bands were founded. Among all studied grain samples, the lower protein bands 5 and 8 were detected in grain samples treated at 10 and 0.5 kGy, respectively (Table 2, Fig. 3). Earlier results showed that grain samples irradiated by 0.5 kGy have good technological properties while grain samples treated at 10 kGy have opposite properties (Błaszczak et al., 2002; Klockiewicz-Kamińska et al., 2000). The former (0.05 kGy) has the higher baking score of 209 and the higher score of bread freshness of 102, while the later (10 kGy) had the lowest baking and freshness scores, respectively, of l76 and 67 from all studied samples. In grain samples treated by 0.05, 0.1 and 1 kGy γ-rays the extracted proteins separated by SDS–PAGE electrophoresis included protein bands which showed the highest molecular masses from 147,780 to 67,095 Da which were not seen in grain samples treated at doses of 0.5, 5 and 10 kGy (Table 2). The lack of the highest molecular mass albumin proteins in these irradiated grain samples suggest that these proteins did not have any or limited influence on technological properties since from all irradiated samples, only grain treated at 0.5 kGy received the highest rating for the following evaluation: wet gluten content, loaf volume, baking score and bread freshness (Klockiewicz-Kamińska et al., 2000). Wheat grain samples γ-irradiated up to 0.5 kGy (Table 2) including the control sample did not change cultivar quality scores and were classified as a top qualify group E (Klockiewicz-Kamińska et al., 2000). The negative influence of γ-irradiation on wheat grain technological properties (Błaszczak et al., 2002; Klockiewicz-Kamińska et al., 2000) was noted in grain samples treated at 1 kGy, where 13 protein bands were detected, up to 10 kGy, with the lowest number of protein bands found (Table 2). It was confirmed earlier (Błaszczak et al., 2002) that γ radiation doses from 1 to 10 kGy cause some structural changes in endosperm microstructure. In addition, Bhatty and MacGregor (1988) suggested the influence of γ-irradiation on the structural alteration of gliadin, which should be also taken into consideration.

4. Concluding remarks

Within the increasing radiation doses, the increase of extractable protein content from wheat grain was observed up to a 5 kGy dose. An increase of reducing sugars content over 100% was noted in all irradiated grain

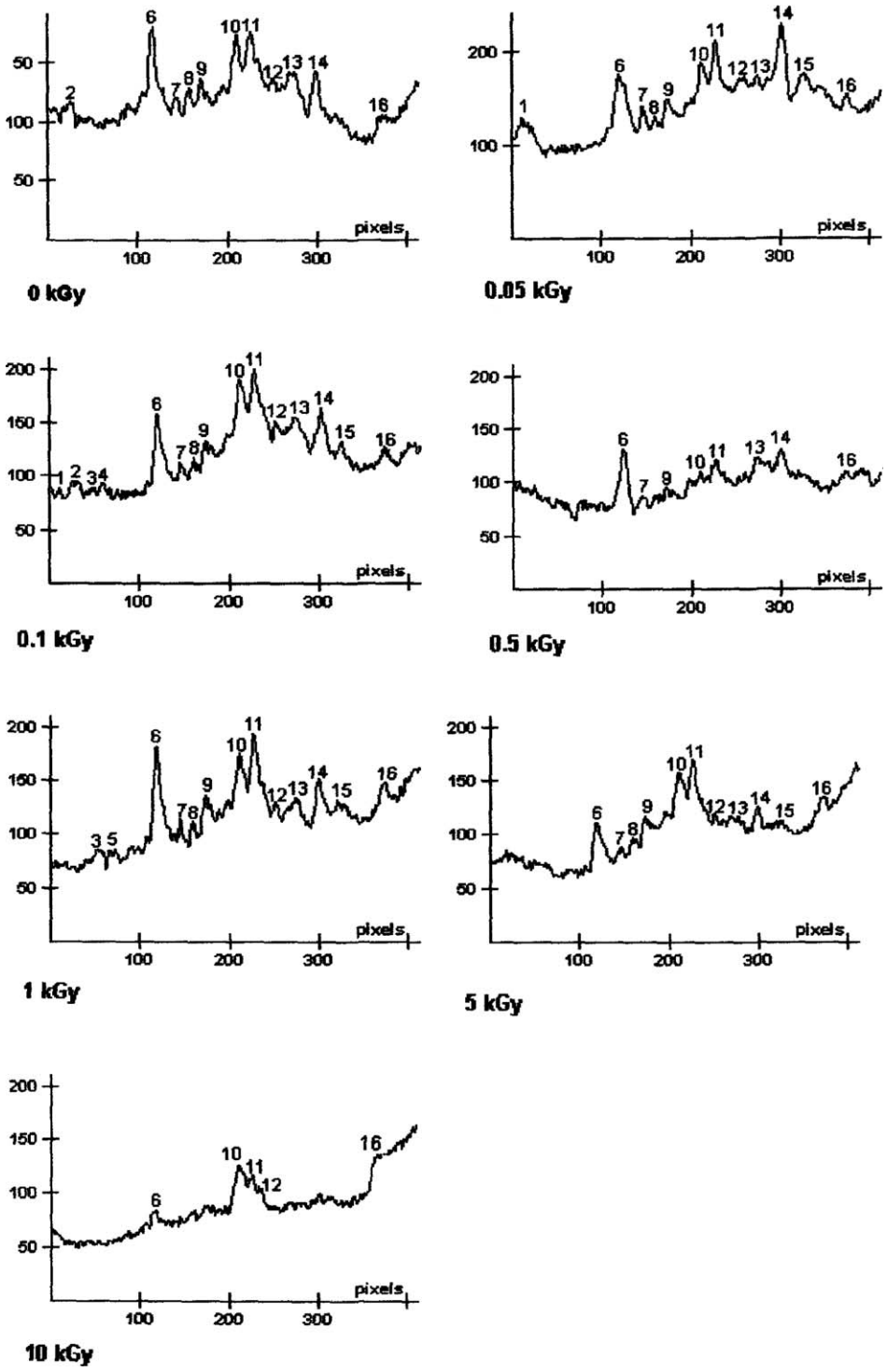

Fig. 3. Densytograms of SDS–PAGE electrophoretically separated albumins from γ-irradiated wheat grain samples.

samples. Ionising radiation increased the activity of wheat grain endogenous amylases (α- and β-amylases), which was statistically significant from the dose of 5 kGy. The increase of amylolitic (α- and β-amylase) activity was well correlated with the highest extractable protein content. Protein extracted from γ-irradiated wheat grain showed a statistically significant increase of inhibition activity against *S. granarius* L. α-amylase at a dose of 0.05 kGy and in the case of *T. confusion* Duv. α-amylase at doses of 0.5 and 1 kGy ($P \leqslant 0.05$) μm. The decrease of inhibition activity against α-amylase of *E. kuehniella* Zell and *S. granarius* L. were noted only at 5 and 10 kGy doses, respectively ($P \leqslant 0.05$). γ-Irradiation doses of 0.05, 0.5 and 1kGy caused an

increase of the antitryptic activity while a dose of 10 kGy decreased this activity ($P \leqslant 0.05$). The highest significant increase of antiamylolitic activity against human saliva α-amylase was noted in albumin proteins of wheat grain treated at doses of 1, 5 and 10 kGy while low doses (<0.5 kGy) caused the decrease of this activity ($P \leqslant 0.05$). In the case of inhibitory activity against hog pancreas α-amylase, the low doses of 0.05 and 0.1 kGy did not change this activity while higher doses (>0.1 kGy) increased this activity ($P \leqslant 0.05$). Etectrophoretic analysis showed among extracted proteins the lowest number of protein bands 5 and 8 in grain samples treated at doses of 10 and 0.5 kGy, respectively. The proteinaceous inhibitors in γ-irradiated wheat grains have shown to have different characteristics.

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