Enzymatic Activity in Wheat Seeds of Different Protein Content

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Summary

We have examined buffer soluble protein content and oxido-reductive and proteolytic enzyme activities of three wheat cultivars with different total protein content. The amount of extracted proteins was lower in wheat with lower total protein content. Activity of glutathione reductase (GR), catalase (CAT), peroxidase (POX) and aspartate protease (phytepsin) was unaffected by differences in total protein content of examined wheat, except in case of peroxidase activity in Srpanjka cultivar. Activity of lypooxigenase (LOX), arginyl and phenylalanyl aminopeptidase (Arg-AP; Phe-AP) was proportionally lower in wheat with lower total protein content. Polyphenoloxidase (PPO) activity was significantly lower, while carboxypeptidase I (CP-I) activity increased with decrease of total protein content. The obtained results indicate that total protein content of wheat affecting the amount of extractable proteins as well as activity of LOX, Arg-AP and Phe-AP, while activities of GR, CAT, POX, PPO, CP-I and phytepsin seem unaffected by total protein content.

Key words

oxido-reductive enzymes, proteolytic enzymes, protein content, wheat

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Introduction

Protein content and quality are among the most important parameters of wheat seed quality. According to total protein content and sedimentation value, wheats were classified in quality groups, while quality and quantity of water insoluble proteins (gliadins and glutenins), play important role in quality grouping of wheat flours (Pomeranz 1971). On the contrary, amount and quality of buffer soluble proteins (albumins and globulins) have still no great importance in wheat or wheat flour classification, although it is well established that this protein fraction affects rheological properties of doughs and quality of final products (Pomeranz 1971, Lásztity 1996).

One of the major components of albumins/globulins influencing rheological properties of doughs and affecting bakery and pasta products quality are enzymes. Among them, the most significant impact have oxidoreductases. Lypoxigenase (LOX), catalase (CAT), peroxidase (POX) and glutathione reductase (GR) positively affect rheological properties of doughs. They have impact on increase of bread volume, improvement of texture and aroma, and negatively affect on carotenoid bleaching causing loss of desirable yellowish pasta color, while polyphenol oxidase (PPO) affect development of unwanted pasta and noodle brownish color (Bettge 2004, Garcia et al. 2000, Fuerst et al. 2006, Kruger et al. 1987, Lásztity 1996, Okot-Kotber et al. 2002, Shiiba 1991, Stauffer 1987). On the other hand proteolytic enzymes of wheat have no significant impact on rheological properties of doughs, but can cause improvement of nutritional quality of final products (Stauffer 1987).

Due to significant effect of enzymes on bakery and pasta products quality, it is evident that enzymes present albumin/globulin quality parameter, and that determination of enzymatic activity in wheat and/or wheat flours could be useful in classification of wheat or wheat flours when requirements for final products quality are used as additional criteria for classification.

Since enzymes belong to albumin/globulin protein fraction which is affected by total protein content (Pratt 1971), it should be expected that increase of total protein cause increase of buffer soluble protein and consequently increase in enzymatic activity.

In order to test this hypothesis we have examined content of buffers soluble proteins, and activity of oxido-reductive and proteolytic enzymes of three wheat varieties with different total protein content.

Materials and methods

Materials

Three winter wheat varieties (*Tritucum aestivum*) from harvest of 2005 were examined in this research. Varieties 'Žitarka' and 'Srpanjka' were cultivated in the fields near Osijek, while 'Divana' near Križevci.

2-naphtylamides of amino acids phenylalanine and arginine, N-carbobenzoxy-L-phenylalanine-L-leucine (CBZ-Phe-Leu), hemoglobin (Hb), guaiacol, sodium 3,5-dichloro-2-hydroxy-benzensulfonate, horseradish peroxidase type VI-A (HRP), ß-nicotinamide adenine dinucleotide 2'-phosphate reduced- tetrasodium salt (NADPH), L-glutathione oxidised-disodium salt (GSSG), 3,4-dihydroxy-L-phenyalanine (L-DOPA) and 2,4,6-trinitrobenzen-1-sulfonic acid (TNBS) were from Sigma (USA). Linoleic acid, 4-aminoantipyrin and sodium tetraborate decahydrate were from Fluka (Germany). All other reagents and solvents were of analytical grade and were used without further purification.

Preparation of extracts

1 g of wheat grains were disintegrated in mortar with pestle using liquid nitrogen, suspended in 5 mL of buffer A (100 mM phosphate buffer pH 7.2 containing 1 mM EDTA-2Na) or buffer B (50 mM sodium acetate buffer pH 5.2 containing 2 mM DTT) and extracted for 1 h at 4°C with vortexing for 30 seconds every 10 minutes. Afterwards extracts were clarified by centrifugation (15000 g, 20 min, 4°C). Obtained supernatants A and B (buffer A, or buffer B) were used for determination of protein content and enzymatic activity. Supernatant A was used for determination of polyphenol oxidase (PPO), catalase (CAT), glutathione reductase (GR) and peroxidase (POX) activity. Supernatant B was used for activity determination of arginyl and phenylalanil aminopeptidase (Arg- and Phe-AP), carboxypeptidase I (CP-I), aspartic proteinase (phytepsin) and lipoxygenase (LOX). Before GR, CAT and POX activity determination, supernatants were additionally purified by removal of interfering substances using PD-10 columns (Amersham, Biosciences).

Enzymatic activity and protein content determination

Aminopeptidase activity was determined by colorimetric method with amino-2-naphtylamides as substrates and Fast Blue B salt as hydrolysis product coupler (Nagatsu et al. 1970). Aspartic protease activity was measured using 2 % (w/v) Hb as substrate (Calucci et al. 2004, Voight et al. 1997). Carboxypeptidase I activity was assayed with 2 mM CBZ-Phe-Leu as substrate and TNBS as hydrolysis product coupler (Mikola 1983, Waters et al. 1980). Catalase activity was determined by colorimetric method with hydrogen peroxide (H_2O_2) as substrate (Fossati et al. 1990). Glutathione reductase activity was measured at 340 nm by continuous spectrophotometric assay of NADPH oxidation as results of GSSG reduction (Torres et al. 1997), PPO activity by dopachrome method at 475 nm with L-DOPA as substrate (Okot-Kotber et al. 2002), POX activity by con-

tinuous spectrophotometric assay at 470 nm with H_2O_2 as substrate and guaiacol as product coupler (Scebba et al. 2001), while LOX activity by continuous spectrophotometric measurement of conjugated diene formation at 234 nm as results of linoleic acid oxidation (Shiiba et al. 1991, Zimmerman and Wick 1970).

Total protein content in grains was determined by near infrared transmission, while soluble protein content by Bradford method (Bradford 1976).

Results and discussion

Total and soluble protein content of wheat varieties

Determination of total protein content in grains of examined wheat showed that varieties differ in total protein content. 'Divana' contained the highest amount, 14.25 %, 'Žitarka' contained 13.37 %, while 'Srpanjka' had the lowest amount, 10.54 %, of total protein. Amount of buffer soluble proteins varied from 7 to 8.3 % of total protein content and was lower in wheat with lower total protein content (Figure 1), except in case of 'Srpanjka' which had higher amount of buffer soluble proteins than 'Žitarka'.

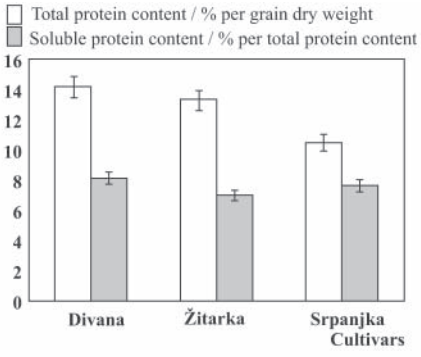

Figure 1. Total and buffer soluble protein content in grains of examined wheat varieties.

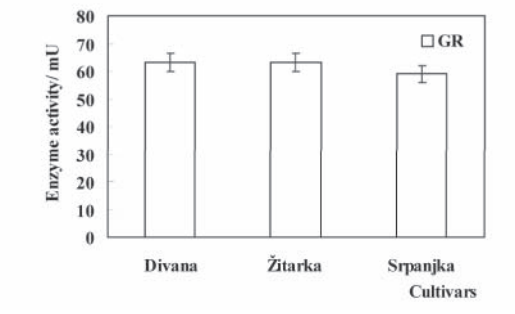

Figure 2. Glutathione reductase activity in extracts of three wheat varieties. Enzyme activity is expressed in mili units (mU). 1U is defined as µmol of released products per minute. Bars present standard error of the mean from 4-6 independent repetitions.

Determined decrease of buffer soluble protein content in varieties with decreased total protein content, as well as noticed differences between varieties in that decrease ('Žitarka', 'Srpanjka') are consistent with previous findings (Pratt 1971), which indicates that differences in amount of buffer soluble proteins among varieties could be attributed to variety characteristics.

Oxido-reductive enzyme activities in wheat cultivars

Oxido-reductive enzyme activity was determined in extracts of dry grains of three wheat cultivars. Glutathione reductase (Figure 2) and catalase activity (Figure 3) was almost the same in all three cultivars. Activities of these two enzymes weren't affected by amount neither of total nor of buffer soluble proteins.

Similarly, activity of peroxidase (Figure 4) was the same for 'Divana' and 'Žitarka', but 'Srpanjka' had lower activity. Contrary to that, activity of lipoxygenase (Figure 5) was proportionally lower, while polyphenol oxidase (Figure 6) activity was significantly lower in wheat with lower total protein content.

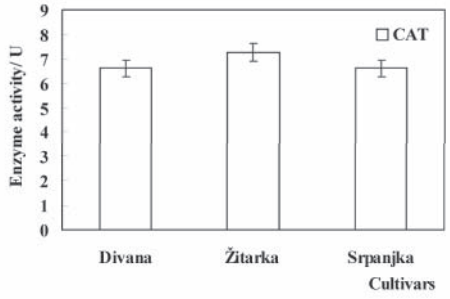

Figure 3. Catalase activity in extracts of three wheat varieties. Enzyme activity is expressed in units (U). 1U is defined as µmol of released products per minute. Bars present standard error of the mean from 4-6 independent repetitions.

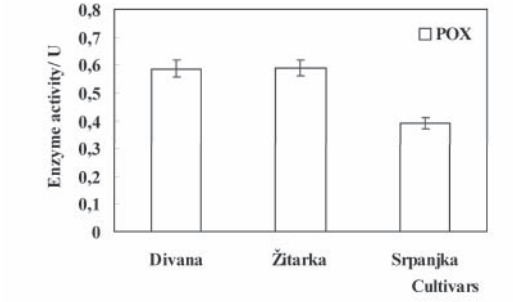

Figure 4. Peroxidase activity in extracts of three wheat varieties. Enzyme activity is expressed in units (U). 1U is defined as µmol of released products per minute. Bars present standard error of the mean from 4-6 independent repetitions.

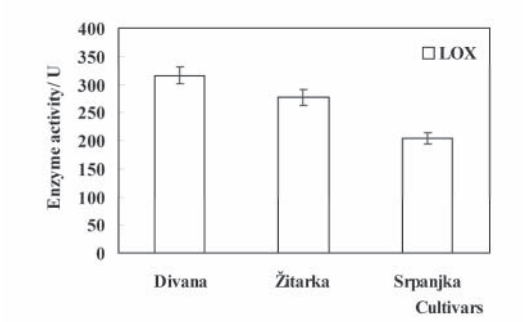

Figure 5. Lipoxygenase activity in extracts of three wheat varieties. Enzyme activity is expressed in units (U). 1U is defined as µmol of released products per minute. Bars present standard error of the mean from 4-6 independent repetitions

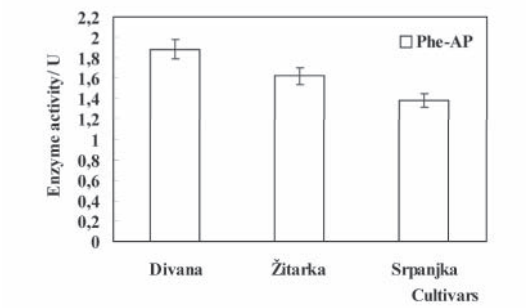

Figure 8. Phenylalanyl aminopeptidase activity in extracts of three wheat varieties. Enzyme activity is expressed in units (U). 1U is defined as µmol of released products per hour. Bars present standard error of the mean from 4-6 independent repetitions

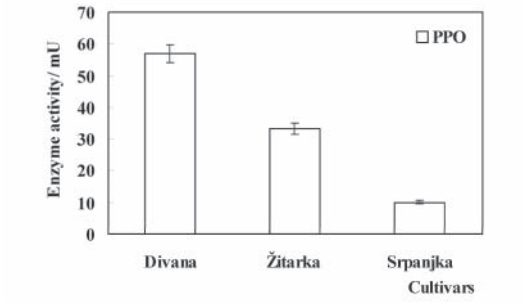

Figure 6. Polyphenol oxidase activity in extracts of three wheat varieties. Enzyme activity is expressed in mili units (mU). 1U is defined as µmol of released products per minute. Bars present standard error of the mean from 4-6 independent repetitions

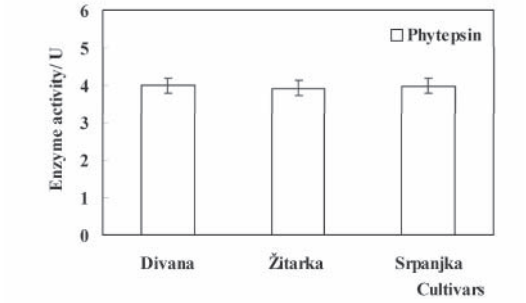

Figure 9. Phytepsin activity in extracts of three wheat varieties. Enzyme activity is expressed in units (U). 1U is defined as µmol of released products per hour. Bars present standard error of the mean from 4-6 independent repetitions

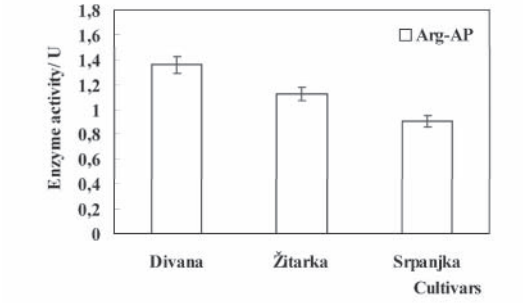

Figure 7. Arginyl aminopeptidase activity in extracts of three wheat varieties. Enzyme activity is expressed in units (U). 1U is defined as µmol of released products per hour. Bars present standard error of the mean from 4-6 independent repetitions

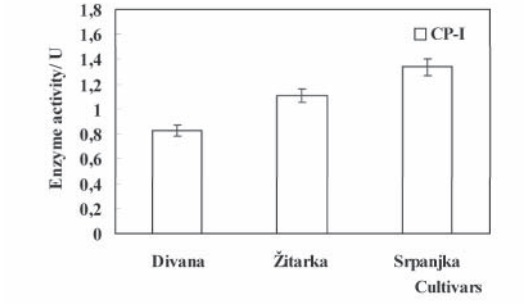

Figure 10. Carboxypeptidase I activity in extracts of three wheat varieties. Enzyme activity is expressed in units (U). 1U is defined as µmol of released products per hour. Bars present standard error of the mean from 4-6 independent repetitions

Although decrease in total protein content cause decrease in amount of buffer soluble proteins of examined varieties, it does not affect activities of all examined oxidoreductases. Activities of glutathione reductase and catalase are unaffected by differences in amount of buffer soluble protein, peroxidase is slightly affected in case of 'Srpanjka', while lypoxigenase activity depends on amount of buffer soluble protein.

This independence of enzyme activity from the amount of buffer soluble protein could be attributed to varietal characteristics, as well as significant differences between varieties in polyphenol oxidase activity.

Proteolytic enzyme activities in wheat varieties

Extracts of dry grains of three wheat varieties were examined for exopeptidase (aminopeptidase and carboxypeptidase) and endopeptidase activity.

Activities of arginyl (Figure 7) and phenylalanyl (Figure 8) aminopeptidase (Arg-2NA and Phe-2NA) were proportionally lower in cultivars with lower total protein content.

Activity of phytepsin was the same in all the cultivars and weren't affected by amount neither of total nor of buffer soluble proteins (Figure 9), while carboxypeptidase I activity increased with decrease of total protein content (Figure 10).

Similarly to oxidoreductases, different proteolytic enzymes show different pattern of dependence of amount of buffer soluble protein. Aminopeptidases are dependent on amount of buffer soluble protein, while phytepsin are not. Independence of phytepsin could be attributed to varietal characteristic as well as observed differences between varieties in carboxypeptidase I activity.

Conclusions

The obtained results indicate that total protein content of wheat affects the amount of extractable proteins as well as activity of LOX, Arg-AP and Phe-AP.

Activities of GR, CAT, POX, PPO, phytepsin and CP-I seem to be unaffected with total or buffer soluble protein content which indicates that activities of these enzymes could be attributed to varietal characteristics.

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