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ANTIOXIDATIVE PROPERTIES OF ALBUMINS IN ENZYMATICALLY CATALYZED MODEL SYSTEMS

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ABSTRACT

The activity of plant origin albumins (obtained from dark and light pea (*Pisum sativum*) seeds and from white and brown bean (*Phaseolus vulgaris*) seeds) and animal origin albumins (ovoalbumin, BSA and human) in 2 enzymatically catalyzed model systems as well as their aromatic hydrophobicity and sulfhydryl groups content were investigated. Pea and bean albumins were much more effective in decreasing the production of superoxide anion radicals in hypoxanthine/xanthine oxidase system (82-97%) than animal-derived preparations (6-26%), whereas no big differences in activity were found when oxidation of linoleic acid by lipoxydase was monitored. No unequivocal correlation between sulfhydryl groups content or aromatic hydrophobicity and the antioxidant properties of preparations was observed.

Key words: legume albumins, antioxidant, enzymatic oxidation, superoxide anion radicals, lipoxydase

INTRODUCTION

Oxidative reactions are one of the most important causes of food shelf life reduction. Many researches are thou concentrated on examining natural antioxidants [10, 13]. Among these proteins were reported to yield antioxidant properties [5], which were often connected to the presence of sulfur and hydrophobic amino acids [8, 2]. However, most of these investigations are based on heat- or transition metal-catalyzed lipid oxidation and stable radicals scavenging, excluding enzymatic catalyzed oxidative reactions, which also play an important role in foods.

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Thus, the aim of this study was the determination of antioxidant properties of legume albumins in comparison with animal-origin albumins in enzymatically catalyzed model systems and the estimation of any correlation of sulfhydryl groups content or aromatic hydrophobicity and the activity of the proteins.

MATERIALS AND METHODS

Albumins preparations were obtained from light and dark varieties of pea (*Pisum sativum*) and white and brown bean (*Phaseolus vulgaris*) by dialysis (cut-off 12 kDa) of alkaline extracts (pH 9.2 for light and 8.0 for dark varieties) prepared from hulled and milled seeds. Precipitated globulin fractions were separated by centrifugation and supernatants were lyophilized. Except legume albumins in the study there were applied animal-derived albumins purchased from Sigma: ovoalbumin, bovine serum albumin (BSA) and human albumin. The preparations' abbreviations used in the study are presented in Table 1.

Table 1. Abbreviations used in the study

PREPARATION	ABBREVIATION USED
white bean seeds albumins	WBA
brown bean seeds albumins	BBA
light pea seeds albumins	LPA
dark pea seeds albumins	DPA
ovoalbumin	OVO
bovine serum albumin	BSA
human albumin	HUA

All preparations were characterized by spectrofluorimetrical measurement (excitation and emission wavelengths 390 nm and 470 nm, respectively) of surface aromatic hydrophobicity after reaction with 8-anilino-1-naftaleno-sulfonic acid (ANSA, Sigma) in methanol [4] and by determination of available sulfhydryl groups content through reaction with 2,2'-dithiobis(5-nitropyridine) (Sigma) and spectrophotometrical assay of products obtained (386 nm) [9, 11]. Absorbances gained in the sulfhydryl groups assay were converted to micromoles SH per gram of protein using calibration curve prepared with glutathione (Sigma).

The antioxidative activity experiments were carried out in 2 model systems. Superoxide anion radicals were generated by hypoxanthine (0.6 mM, Fluka) / xanthine oxidase (20 mIU, Fluka) system in the presence of 2.4 mM DETAPAC (diethylenetriaminepentaacetic acid, Sigma) and 36 mM nitro blue tetrazolium (Sigma) – all solutions in 0.1 M phosphate buffer pH 7.4 [12]. Albumins (100 mg%) were applied in 0.01 M phosphate buffer pH 7.0. Absorbance at 560 nm was measured immediately after xanthine oxidase addition and every 10 min up to 60 min. Initial values were subtracted from all obtained in the assay. Final values were used to calculate the antioxidant activity of the preparations employing the following equation:

$$A_a = (A_c - A_s) / A_c * 100,$$

where:
A_a – antioxidative activity,
A_c – absorbance of control,
A_s – absorbance of sample including proteins investigated.

Efficiency of the preparations towards oxidation of linoleic acid catalyzed by lipoxydase were determined as follows: 10 mg of protein was dissolved in 5 ml 0.01 M phosphate buffer (pH 7.0) and 850 microliters of this solution was mixed with 2 ml of 0.1% linoleic acid (Sigma) in ethanol diluted six fold with 0.2 M borate buffer (pH 9.0). Then 150 microliters of lipoxydase solution (10 000 u/ml of ice-cold borate buffer, Sigma) were added and absorbance at 234 nm was measured after 8 min incubation at room temperature against protein blank. Controls were prepared with 850 microliters of phosphate buffer instead of protein solution. Antioxidant activity of the preparations were calculated using the above equation.

All the experiments were performed in at least 3 replications. Means and standard deviations were calculated using Microsoft Excel 2000 and correlations were estimated using Statgraphics Plus 2.1.

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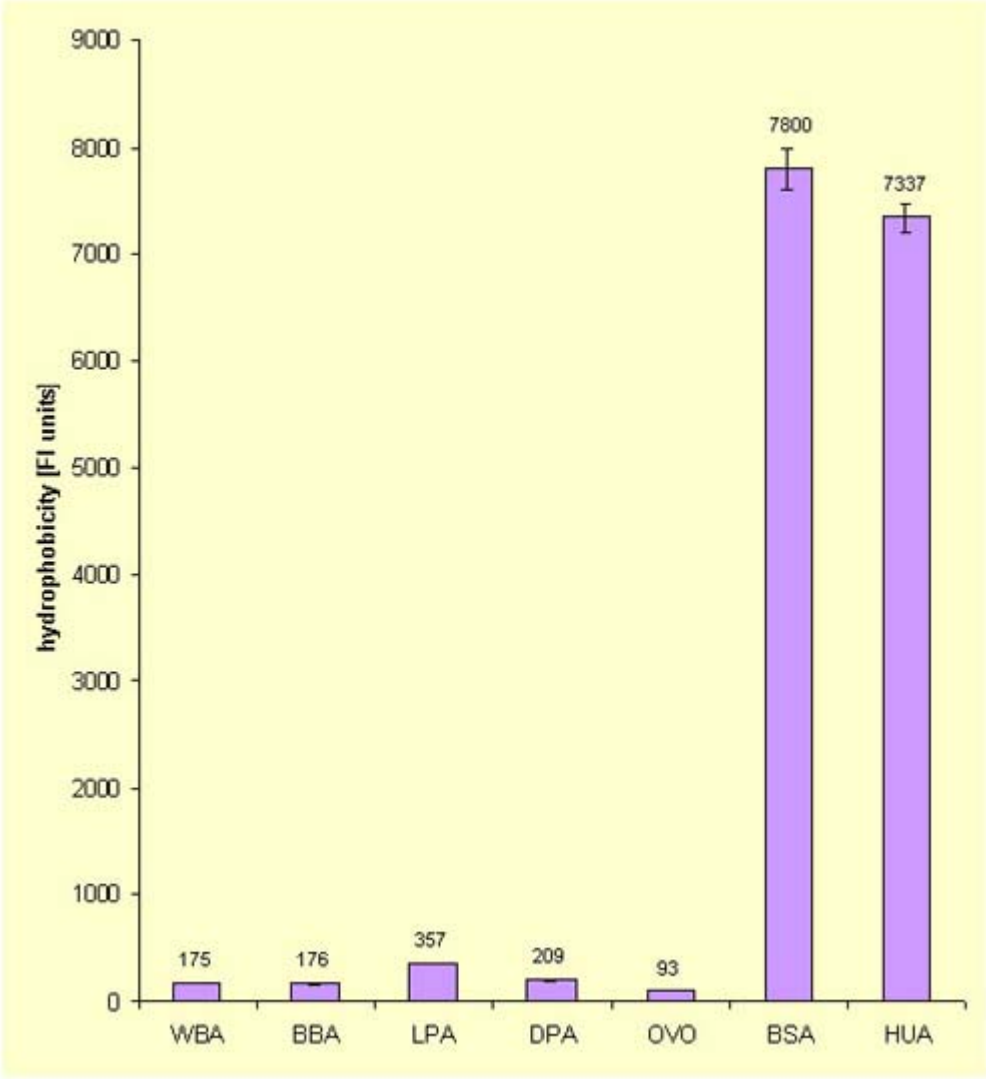
RESULTS AND DISCUSSION

Plant-derived albumins had quite low surface aromatic hydrophobicity (Fig. 1). Bean albumins were characterized by a little smaller values, but showing no difference between the 2 varieties (175 and 176 FI units), whereas light pea albumins had bigger hydrophobicity (357 FI units) than the dark one (209 FI units). Among animal preparations ovoalbumin was the least hydrophobic in this study (93 FI units), but had the same order of magnitude as legume proteins tested. On the contrary, BSA and human albumin were characterized by incomparably higher values of hydrophobicity (7800 and 7337 FI units, resp.). Although not fully comparable, these results stand in agreement with the work of Kato et al. [6] showing very little hydrophobicity of ovoalbumin (cis-parinaric acid probe), clearly higher in case of soy globulin and significantly higher for BSA. Our findings are also well-correlated with those of Haskard and Li-Chan [3], where surface aromatic hydrophobicity of BSA was about 280 times higher comparing to ovoalbumin.

Fig. 1. Surface aromatic hydrophobicity of the albumin preparations



No such big differences were found in available sulfhydryl groups content (Fig. 2). Ovoalbumin and especially BSA yielded smaller values (13 and 7 micromoles SH/g protein, resp.) than pea and bean albumins (16-40 micromoles SH/g protein). Human albumin as the only from animal albumins investigated had similar sulfhydryl groups content to legume albumins (26 micromoles SH/g protein). These results confirm other authors' findings indicating relatively high sulfur amino acids content in legume albumins [1, 7].

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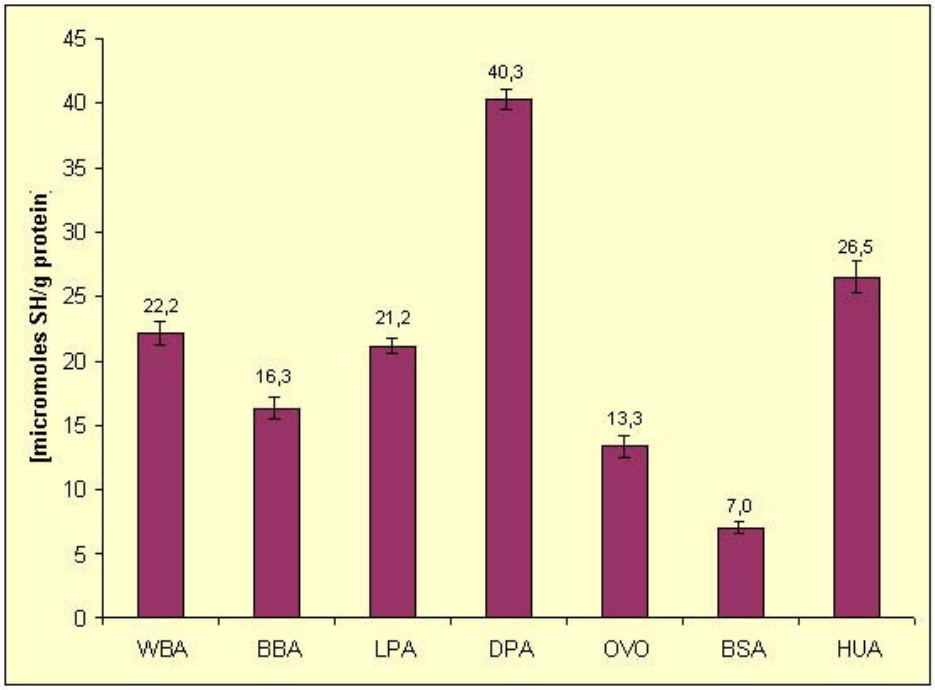
Fig. 2. Sulfhydryl groups content of the albumin preparations



In the first oxidative experiment activity of hypohanthine/xanthine oxidase system resulted in superoxide anion radicals production causing reduction of nitro blue tetrazolium and gain of absorbance at 560 nm (Fig. 3 and 4, black curve). Addition of pea and bean proteins considerably reduced the amount of superoxide anion radicals keeping the absorption values very low (Fig. 3), but applying animal-origin albumins did not bring about such a substantial result, showing their very moderate activity (Fig. 4). These statements are confirmed by antioxidant activity values calculated for the final absorbances in this assay (Fig. 5). All the legume albumins were characterized by an activity higher then 82%, among which bean proteins were better antioxidants in this model system (93-97%). Results obtained in this experiment for pea and bean albumins are very similar to the findings of Wettasinghe and Shahidi received for evening primrose meal extract [12].

Fig. 3. Influence of legume albumin preparations addition on superoxide anion radical content

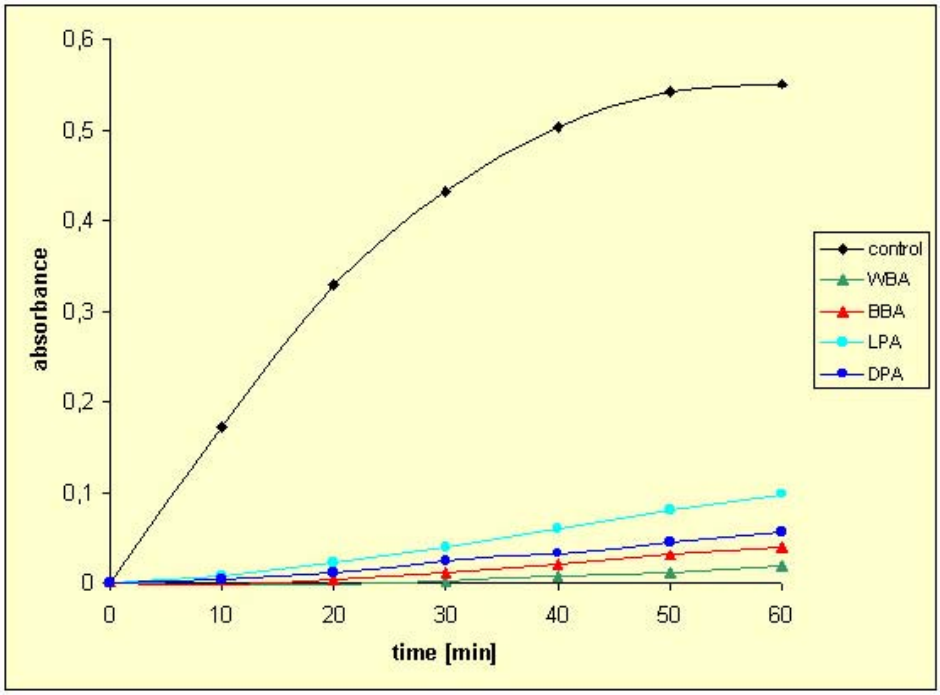


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Fig. 4. Influence of animal albumin preparations addition on superoxide anion radical content

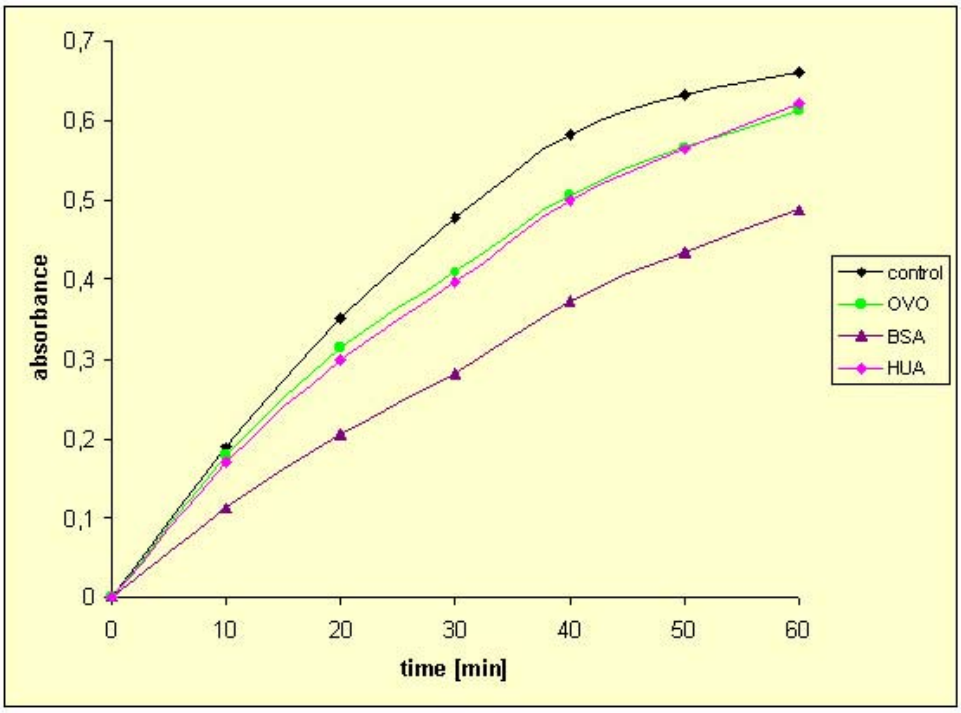
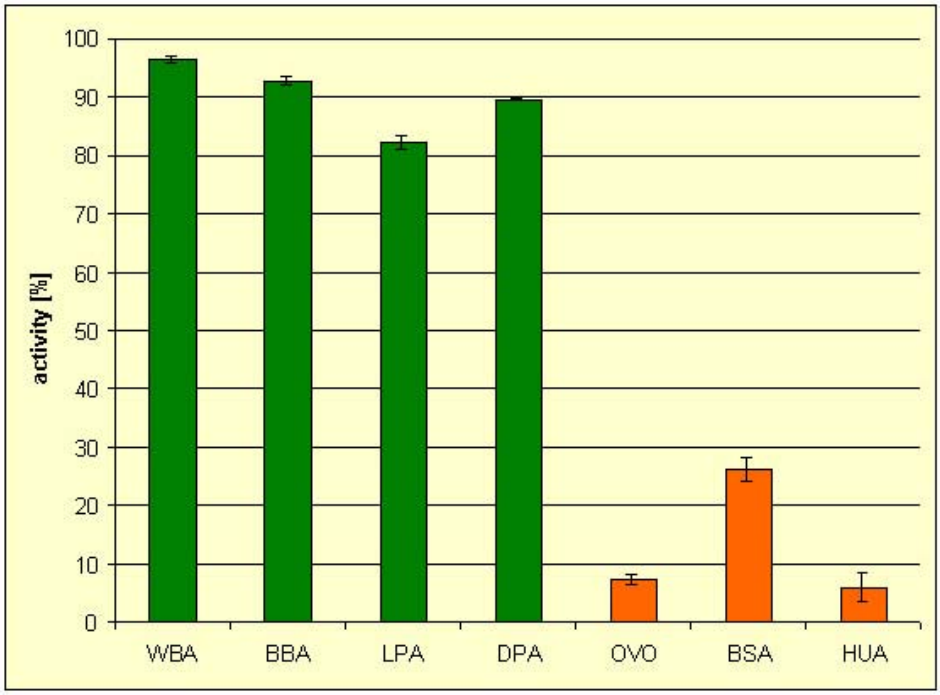


Fig. 5. Albumin preparations antioxidant activity against superoxide anion radicals generated in HPX/XOD system



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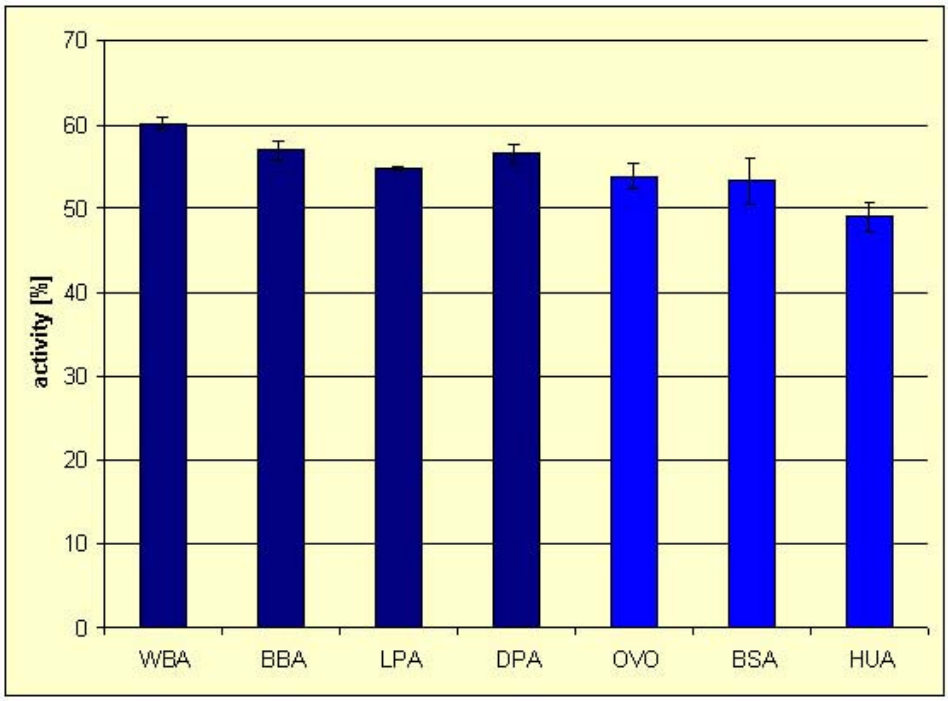
In the second model system we employed a direct reaction of linoleic acid oxidation by lipoxydase. The process was monitored spectrophotometrically at 234 nm. On the contrary to the previous experiment, all the preparations examined showed good antioxidant properties and no big differences in efficiency was found (Fig. 6). Although animal-derived albumins exhibited worse properties in this assay (49-54%), pea and bean albumins were only slightly more active (55-60%). One of the possible explanations of this phenomena is the difference of antioxidative action in the experiments described above. The second model system employs fatty acid which does not dissolve in water, so proteins may be located on the interphase and their activity may be depending much more upon creation a physical barrier than upon any chemical reaction. This lowers the meaning of the chemical structure differences increasing the importance of physical properties.

Fig. 6. Activity of the albumin preparations against linoleic acid oxidation caused by lipoxydase



No unequivocal correlation between sulfhydryl groups content or aromatic hydrophobicity and the antioxidant properties of preparations was found in both cases. On the other hand there can be noted a small positive influence of SH groups content at the activity of legume albumins preparations concerning only samples of the same species. This may suggest some impact, but also importance of other factors related to the structure of these proteins.

CONCLUSIONS

1. All the albumin preparations employed in the study exhibited antioxidative properties.
2. Pea and bean albumins were more efficient antioxidants comparing to the preparations of animal origin.
3. No correlation between sulfhydryl groups content or aromatic hydrophobicity and the activities of the preparations was proved.

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