POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

Pol. J. Food Nutr. Sci. 1998, Vol. 7/48, No 2

APPLICATION OF RAMAN SPECTROSCOPY TO STUDY THE HIGH PRESSURE EFFECT ON THE SECONDARY STRUCTURE OF BEAN (*PHASEOLUS VULGARIS*) AND PEA (*PISUM SATIVUM*) PROTEINS

Mirosława Klepacka, Krzysztof Bajdor[1], Artur Jeżewski[2]

Division of Food Quality Control, Faculty of Food Technology, Warsaw Agricultural University, [1]Industrial Chemistry Research Institute, Warsaw, [2]Institute of Organic Chemistry of the Polish Academy of Sciences, Warsaw.

Key words: Raman spectroscopy, leguminose proteins, high pressure, secondary structure.

The effect of high pressure on the secondary structure of bean and pea proteins was studied using Raman spectroscopy. The proteins were pressurized at 300 and 600 MPa. Raman spectra were obtained for samples both in the solid state and dissolved in D_2O. The results indicated that the contributions of unordered structure in pressurized proteins increased at the expense of the contributions of the α-helix and β-structure. High pressure denaturation produced more changes in β-structure of pea proteins than bean proteins. Intensity analysis of band at 853 and 828 cm^{-1} showed that the residues of tyrosine were buried in the pressurized bean proteins, whereas in pea proteins the residues of tyrosine were exposed on the surface of protein molecules by high pressure. The results indicated that upon pressure processing the proteins of bean and pea folded and unfolded respectively. The Raman spectra of bean proteins in solid state revealed differences in comparison with the proteins solution in D_2O. These changes were observed in the intensity of the bands in the region of 1000 to 1350 cm^{-1}. The extent of changes in the secondary structure of proteins in the solid state was smaller in comparison with the deuterated samples after denaturation by high pressure.

INTRODUCTION

The effect of high pressure on the changes in the protein secondary structure is not univocally discussed in the literature. Generally, it is considered that the secondary structure is destroyed above 1000 MPa [Masson, 1992; Taniguhi & Takeda,

Author's address for correspondence: Mirosława Klepacka, Zakład Oceny Jakości Żywności, Wydział Technologii Żywności, SGGW, Rakowiecka 26/30, 02-528 Warszawa, tel. (48 22) 491924; fax (48 22) 491375

1992; Wong & Heremans, 1988; Wong *et al.*, 1989]. Some proteins treated at 300 MPa and 600 MPa showed only little effect on the secondary structure changes [Kim *et al.*, 1996; Heremans & Heremans, 1989]. The structures of myoglobin and chymotrypsin were changed, however, under similar range of high pressure [Heremans & Heremans, 1989; Taniguhi & Takeda, 1992].

In literature, several aspects were reported which affect the changes in protein structure. Among these factors various conditions, such as pH enviroment, the protein concentration, as well as the extent of secondary structure rearrangements [Carrier *et al.*, 1990; Heremans & Heremans, 1989; Silva *et al.*, 1989] were given.

Generally, the studies on the effect of pressure on the structure of proteins have been undertaken with milk and meat proteins [Carballo *et al.* 1996, 1997; Cheftel, 1996; Dickinson & Pawlowsky, 1996; Iametti *et al.*, 1997; Jegouic *et al.*, 1997; Kim *et al.*, 1996; Lopez-Fandino *et al.*, 1996; Perez-Mateos *et al.*, 1997; Stapelfeldt *et al.*, 1996]. However, the number of pressure studies in the literature on the structural changes of vegetable proteins is rather small when compared with another proteins [Gekko, 1992; Hayashi, 1992].

The application of high pressure in the food processing on a larger scale requires the investigation of their effects on the vegetable proteins.

The changes in the protein secondary structure, caused by the denaturation, affect their physico-chemicaland functional properties, which are significant in the food processing technology.

In this study Raman spectroscopy was applied to investigate the effect of high pressure on the secondary structure of bean and pea proteins. Moreover, the effect of heat treatment on these proteins was also examined.

MATERIALS AND METHODS

MATERIAL: The legume protein samples were prepared from the flour of bean [*Phaseolus vulgaris*] var. Mela and pea [*Pisum sativum*] var. Poa. The samples were obtained by isoelectic precipitation from basic (pH 9.2) extracts of flour [Klepacka & Porzucek, 1994]. Proteins were recovered by centrifugation at 5500 × g for 20 min at 4°C. The precipitates were re-dispered in distilled water, adjusted to pH 7.0 and lyophilized.

METHODS: The Raman spectra were recorded on Perkin-Elmer spectrometer system 2000 equipped with the Raman accessory. The excitation source was Nd:YAG laser (1064 nm). Typical parameters of the measurement were as follows: spectral resolution of 4 cm^{-1}, 256 scans for each spectrum, laser power below 300 mW, the detection geometry 180°. The measurement was conducted in a glass capillary. The Raman spectra in the range from 600 to 1800 cm^{-1} were obtained for samples in D_2O solution and in the solid state (only bean protein). Deuterium oxide (minimum 99.9% D atom) was obtained from the Institute of Atomic Energy, Isotope Production and Reactor Centre (Otwock-Swierk, Poland). In the preliminary experiments the protein concentration in D_2O solution was estimated. From these experiments it followed that the satisfactory results were obtained with the solu-

tion of 120 mg/mL and 150 mg/mL of pea and bean proteins, respectively. The protein solutions were prepared by sample extraction in deuterium oxide for 15 min on a microshaker. After centrifugation (8000 × g, 15 min) the supernatants were placed in the teflon tubes for the high pressure treatment. The tubes were made according to the prototype by Jurczak and Gryko [1996]. For heat-denaturation study the supernatants were sealed into glass tubes and heated for 30 min by immersion in a boiling water bath. The protein samples were pressurized using a LCP-20 apparatus at 300 and 600 MPa for 30 min at 20°C. High pressure treatments of the samples in the solid state were carried out at 600 and 1000 MPa. According to Williams and Dunker [1981] and Williams [1983], the Amide I band was used to calculate the protein secondary structure content. The Amide I component bands at 1642, 1670 and 1650 cm^{-1} were due to the peptide residues in α-helix, β-sheet (antyparallel) and unordered structure, respectively. Using the computer program GRAMMS (GALACTIC Ind.Corp.) the Amide I band was resolved into the Lorentzian components. The data obtained by these methods enabled the comparison of the area of the band assigned to particular structure. The intensity of the band at 1448 cm^{-1} assigned of CH_2 groups present in the proteins were used as an internal standard. [Twardowski & Anzenbacher, 1988]. All analyses were performed three times and the results were reported as average of these replicates.

RESULTS AND DISCUSSION

The interpretation of the Raman spectra used in this work was based on the data of the analyses developed by Areas *et al.* [1989], Li Chan and Nakai [1991], Nonaka *et al.* [1993] and Twardowski and Anzenbacher [1988].

In the preliminary analyses, the proteins were solubilized in water. However in aqueous solution the Amide I vibrations were obscured by the water bending mode and so could not be used for identification of protein structure in water solution. Using the deuterium oxide solutions uncovered the Amide I band. The exchange of hydrogen atoms to deuterium atoms resulted in more stable of deutered bonds comparing to those of containing hydrogen. In the result of this process, hydrogen atoms in the OH^- and N-H groups were replaced by deuteriun atoms, but the exchanges did not occur for the >CH-group.

In the Raman spectroscopy information on the protein structure arises from the Amide I region, because the Amide II mode is Raman inactive.

The Raman Amide I bands were centered around 1660 cm^{-1}. The Amide I band in laser Raman spectroscopy of protein is the sum of the individual bands due to several types present in the protein secondary structure.

The Amide I vibration shifted to lower frequencies and this behaviour was consented with the rate of pressure. The frequency of Amide I band shifted by 1 and 2 cm^{-1} towards lower frequency at 300 and 600 MPa, respectively. These tendencies were observed in all samples. It is attributed to the changes in the conformation of proteins due to the changes of hydrogen bonding with the C=0. When hydrogen bonding interaction effects increased, the Amide I band shifted towards lower frequencies.

TABLE 1. Pressure effect on the changes of the secondary structure of bean and pea proteins.

Sample	Percentage of secondary structure			
	α-Helix	β-Sheet	Unordered	Undefined
Bean :				
Control	32	22	31	15
Pressured at:				
300 MPa	27	20	43	10
600 MPa	23	26	40	11
Heated:				
100°C /30 min	21	12	55	12
Pea :				
Control	29	28	35	8
Pressured at:				
300 MPa	25	18	46	11
600 MPa	20	17	51	12
Heated				
100°C /30 min	18	34	41	7

TABLE 2. Ratio of the relative intensities of the band at 981 cm^{-1} cm to at 1450 cm^{-1} on the Raman spectrum

I 981 cm^{-1}/ I 1450 cm^{-1}				
Sample	Treatment			
	Control	Pressured at (MPa)		Heated
		300	600	100°C/ /30 min
Bean	0.26	0.29	0.33	0.40
Pea	0.26	0.30	0.34	0.36

Estimation of the changes of the protein secondary structure induced by high pressure and heating were carried out by computer-aided analysis with regard to Lorentzian deconvolution. The results are presented in Table 1. Quantitative estimation of the relative amounts of α-helix, β-sheet, and unordered structures as well as unidentifited bands in the range 1615–1604 cm^{-1} was carried out by comparison of relative integrated area of these individual types of the structure.

In the deuterated bean and pea proteins (unpressurized control samples) the content of unordered structure was about 30%. After high pressure treatment the changes in secondary structure content were observed. There was a decrease in α-helix and β-structure accompanied by an increase of unordered structure. After heat treatment these changes were more pronounced. The differences between the content of α-helix structure in the control samples and heated ones were 11% while in the pressurized proteins the decrease of the α-helix structure did not exceed 10%.

In the pressurized pea proteins the β-structure decreased by 10–11% in the secondary structure. In the bean proteins no significant changes in β-structure took place. In the pressure of 600 MPa there was even slow increase.

After heat treatment of bean proteins, virtually, a loss of β-structure was observed, whereas the heat denaturation of pea proteins resulted in an opposite change to that of bean. In these samples the contribution of β-structure increased in comparison with control samples. The increase of β-structure fraction can be explained by the changes leading to the gel formation. Although, these kinds of changes were not observed in our investigation, it indicates, that the changes in pea proteins as induced by heating differ from those in bean proteins. Also the considerable increase of unordered structure in heat-denaturated bean proteins was confirmed by these differences. Whereas in heat-denaturated pea protein the changes in the unordered structure were smaller when compared to pressurized proteins.

Also a band characteristic for an unordered structure which appeared in Raman spectra at 981 cm^{-1} was investigated. The ratio of the band intensity at

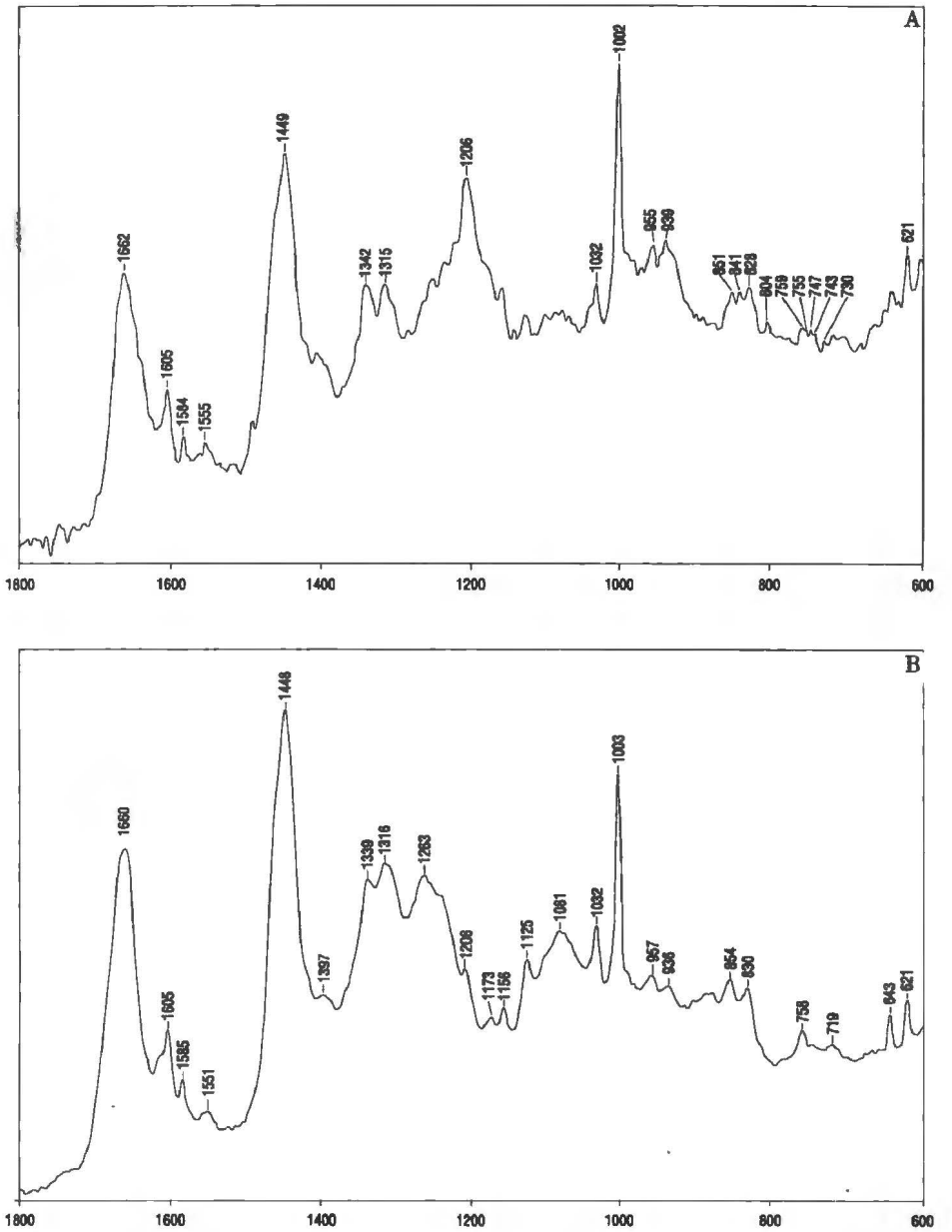

FIGURE 1. Raman spectra of bean proteins (control samples) in solution (A) and solid state (B). Solution conditions: protein concentration 15 mg/mL in D_2O.

981 cm^{-1} to 1449 cm^{-1} (internal standard) increased in both samples, pressurized and heated, in comparison with the native ones (Table 2). In the heated proteins this ratio was higher than for pressurized samples. As can be seen, the pressurization revealed also changes in the unordered conformation. This ratio increased with pressure. These results confirmed the information given earlier in this paper.

TABLE 3. Pressure effect on the Raman spectrum of the secondary structure of the bean protein in the solid state.

Sample	Percentage of secondary structure			
	α-Helix	β-Sheet	Unordered	Undefinited
Control	48	30	11	11
Pressured at:				
600 MPa	47	29	12	10
1000 MPa	54	23	15	8
Heated:				
100°C/30 min	50	25	15	11

The denaturation changes in the pressurized proteins were also studied by Raman spectra of solid state samples. Only the bean protein samples were investigated.

The examples of Raman spectra of control bean protein in D_2O solution and solid state are shown in Fig. 1. As can be seen some evident changes occurred in these spectra. The intensities of the band in the range 1000-1350 cm^{-1} were altered in both spectra. The spectrum of bean proteins in D_2O solution, revealed the presence of the band around 1200 cm^{-1}, typical of D_2O, attributed to the bending vibration, which impeded the analysis. But, one can also observe a strongly exposed band centered at 1206 cm^{-1}. The intensity of this band of solid sample spectrum was smaller, but the band at 1263 cm^{-1} was stronger exposed. The band spectrum of D_2O protein solution at 1263 cm^{-1} of smaller intesity was observed. The bands at 1081 cm^{-1} and 1125 cm^{-1} in the spectrum of solid sample were stronger than those of deutered proteins.

TABLE 4. Ratio of the relative intensities of the band at 853 cm^{-1} to at 828 cm^{-1} (S) on the Raman spectrum.

I 853 cm^{-1}/ I 828 cm^{-1} (S)				
Sample	Treatment			
	Control	Pressured at (MPa)		Heated
		300	600	100°C/ /30 min
Bean	0.90	0.86	0.82	0.67
Pea	0.65	0.88	0.87	1.10

TABLE 5 . Ratio of the relative intensities of the band at 1340 cm^{-1} to at 1449 cm^{-1} and of the band at 1003 cm^{-1} to at 1449 cm^{-1} on the Raman spectrum.

I 1340 cm^{-1}/ I 1449 cm^{-1}				
Sample	Treatment			
	Control	Pressured at (MPa)		Heated
		300	600	100°C/ /30 min
Bean	0.35	0.34	0.31	0.24
Pea	0.31	0.31	0.30	0.38
	I 1003 cm^{-1} / I 1449 cm^{-1}			
Bean	0.92	0.93	0.85	0.76
Pea	0.70	0.69	0.70	0.61

The data on effect of high pressure on changes of pressurized bean proteins in solid state are shown in Table 3. In the solid state there are larger contributions of α-helix structure than in protein solution in D_2O. Lippert *et al.* [1976] suggested that a decrease in structure-helix was, accompanied by dissolution. Our observations confirmed the Lippert's study. The results in Table 3 indicate that the pressurization of the protein in solid state (without water) caused smaller changes of intensity than in the deuterated samples. No significant changes in formation of unordered structure were evident after high pressure treatment of solid samples. Our data confirm the study of chymotrypsinogen [Heremans & Heremans, 1987].

The additional information about the denaturation changes in the amide bands can be obtained from the interaction of amino acid residue with one another and with the enviroment. Mainly there are aromatic amino acids, which have characteristic bands in the

Raman spectra of protein. Positions of Raman bands of aromatic acids sensitive to conformational changes have the following frequencies:

tyrosine 850, 830 and 645, tryptophan 1363 and phenylalanine 1005, 622.

The intensity ratio of the bands (S) of tyrosine at about 855 and 835 cm^{-1} was used to describe the extent of hydrogen bonding between the OH^- residues and enviroment in the protein. In the native protein tyrosine is burried in protein molecule, but for unfolded protein the tyrosine residues are exposed on the molecular surface and interact with the hydrophilic medium.

The doublet at 855 cm^{-1}/835 cm^{-1} arises from a Fermi resonance. The changes induced by the denaturation factors influence the nature of hydrogen bonding and variation of intensity ratio of this doublet. In the native protein molecules the band at 830 cm^{-1} predominates over the band at 850 cm^{-1}, but in the denaturated protein the opposite situation is observed. In the Raman spectra of deuterated bean and pea proteins the bands of tyrosine were at 853 cm^{-1} and 828 cm^{-1}. The intensity ratio (S) of the doublet 853 cm^{-1}/828 cm^{-1} is shown in Table 4. As can be seen, in the pressurized bean proteins the ratio decreased. The effect of heating on the doublet ratio was larger than for the corresponding pressurized samples. The decreasing of doublet ratio suggests that in the pressurized and heated bean proteins tyrosine residues were more involved in weak hydrogen bond between the OH^- group and the environment. The effect of "burriedness" of tyrosine was more intense in heated protein than in pressurized one. Whereas the opposite changes in pea proteins were observed. The pressure- and temperature-induced denaturation of the pea proteins affected the increase of doublet ratio. This can lead to a conclusion that the changes in intensity ratio indicate unfolding of the structure of the pea proteins and exposing of tyrosine residues on the surface of molecules. Significantly stronger changes in pea proteins were observed when proteins underwent heat denaturation with larger exposition of tyrosine residues. After the treatment at 300 and 600 MPa similar changes appeared in pea proteins.

The band at 1340 cm^{-1} in the Raman spectra of pea and bean proteins was assigned to the deutered tryptophan residues. In Table 5 intensity ratio between bands at 1340 cm^{-1} and 1449 cm^{-1} (internal standard) are shown. The 1340 cm^{-1}/1449 cm^{-1} intensity ratio did not change in the pressurized proteins. The situation is changed for heated samples. The ratio decreased in bean proteins but increased in pea proteins.

In our spectra, no band at 1360 cm^{-1} was observed in bean and pea proteins.

The heated pea and bean proteins were more sensitive to such changes than the pressurized samples.

In the spectra observed the predomination of bands at 1002 cm^{-1} assigned to the phenylalanine correlated with breathing vibration of the benzene ring.

The intensity ratio between bands 1002 and 1449 cm^{-1} (Table 5) was calculated. A distinct changes of decreasing the band intensity assigned to the phenylalanine occurred in the heated bean and pea proteins. No significant intensity changes in the spectra of pressurized proteins were observed. The band intensity was decreased only in the bean protein pressured at 600 MPa.

The results obtained in present studies indicate, that the pressurization induced the denaturation changes in the secondary structure of the proteins.

CONCLUSIONS

1. The secondary structure of the deuterated bean and pea proteins changed upon high pressure treatment at 300 and 600 MPa, as inferred by Raman spectroscopy.

2. The changes in the structure were dependent on the rate of high pressure and the nature of the proteins.

3. The decrease of β-structure was observed in the pressure-induced pea proteins, while in the bean proteins the decrease of α-helix structure was more pronounced.

4. The pressurized proteins in the solid state exhibited smaller changes of the secondary structure than soluble proteins.

5. Conformation changes of the pressure-induced denaturation of bean and pea proteins differ considerably from those of the temperature-induced denaturation of the same proteins.

6. The changes in the Raman bands related to the vibrations of tyrosine residues exhibited differentions of the behaviours in the pressure-induced proteins caused by unfolding of the pea proteins and involved of the bean proteins.

REFERENCES

1. Areas E.P.G., Laure C.J., Gabilan N., Araujo P.S., Kawano Y., Raman and infrared studies on the conformation of porcine pancreatic and *Crotalus durissus terrificus* phospholipases A_2. Biochim. Biophys. Acta, 1989, 997, 15–26.
2. Carballo J., Fernandez P., Colmenero F.J., Texture of uncooked and cooked low- and high-fat meat batters as affected by high hydrostatic pressure. J. Agric. Food Chem., 1996, 44, 1624–1625.
3. Carballo J., Fernandez P., Carrascosa A.V., Solas M.T., Colmero J., Characteristics of low – and high-fat beef patties: Effect of high hydrostatic pressure. J. Food Protection, 1997, 60, 48–53.
4. Carrier D., Mantsch H., Wong P.T.T., Pressure-induced reversible changes in secondary structure of poly- (L-lysine):An IR spectroscopics study. Biopolymers, 1990, 29, 837–844.
5. Cheftel J.C., Effects of high pressure on meat: a review. 42nd ICoMST- Lillehammer, Norway, 1–6 September 1996, 1–30. Manuscript.
6. Dickinson E., Pawlowsky K., Effect of high – pressure treatment of protein on the rheology of flocculated emulsions containing protein and polysaccharide. J. Agric. Food Chem., 1996, 44, 2992–3000.
7. Gekko K., Effects of pressure on the sol-gel transition of food macromolecules, 1992, *in:* High Pressure and Biotechnology, vol. 224 (ed. Balny C., Hayashi R., Heremans K., Masson P.) Colloque INSERM/John Libbey Eurotext Ltd., pp. 105–113.
8. Hayashi R., Utilization of pressure in addition to temperature in food science and technology, 1992, *in:* High Pressure and Biotechnology, vol. 224 (ed. Balny C., Hayashi R., Heremans K., Masson P.) Colloque INSERM/John Libbey Eurotext Ltd, pp. 185–193.
9. Heremans L., Heremans K., Raman spectroscopic study of the changes in secondary structure of chymotrypsin: effect of pH and pressure on the salt bridge. Biochem. Biophys. Acta, 1989, 999, 192–197.
10. Iametti S., Transidico P., Bonomi F., Vecchio G., Pittia P., Rovere P., Dall Aglio G., Molecular modifications of β-lactoglobulin upon exposure to high pressure. J. Agric. Food Chem., 1997, 45, 23–29.

11. Jegouic M., Grinberg V.Y., Guingant A., Haertle T., Baric oligomerization in α-lactoalbumin /β-lactoglobulin mixtures. J. Agric. Food Chem., 1997, 45, 19–22.
12. Jurczak J., Gryko D., 1996 Organic synthesis at high pressure. *in*: Chemistry under Extreme or Non-classical Condition. (ed. Rudi Van Eldik, Colin D. Hubb) John Wiley & Sons, Inc. and Spectrum, Akademischer Verlag Co-Publication, New York, Heidelberg, pp. 163–189.
13. Kim K., Ikeuchi Y., Suzuki A., Pressure effects on circular dichroism spectra of connectin. Biosci. Biotech. Biochem., 1996, 60, 1488–1489.
14. Klepacka M., Porzucek H., Some properties of chemically modified bean, lupin and pea proteins. Pol. J. Food Nutr. Sci., 1994, 2/44, 3, 45–46.
15. Li-Chan E., Nakai S., Raman spectroscopic study of thermall and/or dithiothreitol induced gelation of lysozyme, J. Agric. Food Chem., 1991, 39, 1238–1245.
16. Lippert J.L., Tymiński D., Desmeules P.J., Determination of the secondary structure of proteins by laser Raman spectroscopy. J. Amer. Chem. Soc., 1976, 98, 22, 7075–7080.
17. Lopez-Fandino R., Carrascosa A.V. Olano A. The efeects of high pressure on whey protein denaturation and cheese-making properties of raw milk. J. Dairy Sci., 1996, 79, 929–936.
18. Masson P., Pressure denaturation of proteins, 1992, *in*: High Pressure and Biotechnology, vol. 224 (ed. Balny C., Hayashi R., Heremans K., Masson P). Colloque INSERM/ John LibbeyEurotext Ltd., pp. 89–99.
19. Nonaka M., Li-Chan E., Nakai S., Raman spectroscopic study of thermally induced gelation of whey proteins. J. Agric. Food Chem., 1993, 41, 1176–1181.
20. Perez-Mateos M., Lourenco H., Montero P., Borderias A.J. Rheological and biochemical characteristics of high-pressure and heat-induced gels from Blue Whiting (*Micromesistius poutassou*) J. Agric. Food Chem., 1997, 45, 44–49.
21. Silva J.L., Villas-Boas M., Bonafe F.S., Meirelles N.C., Anomalous pressure dissociation of large protein aggregates. J. Biol. Chem., 1989, 264, 15863–15868.
22. Stapelfeldt H., Petersen H., Kristiansen K.R., Qvist K.B., Skibsted L.H., Effect of high hydrostatic pressure on the enzymic hydrolysis of β-lactoglobulin B by trypsin, thermolysin and pepsin. J. Dairy Res., 1996, 63, 111–118.
23. Taniguchi Y., Takeda N., Pressure-induced secondary structure of proteins studied by FT-IR spectroscopy, 1992, *in*: High Pressure and Biotechnology, vol. 224 (ed. Balny C., Hayashi R., Heremans K., Masson P.) Colloque INSERM/John Libbey Eurotext Ltd., pp. 115–122.
24. Twardowski J., Anzenbacher P., 1988, Spektroskopia Ramana i podczerwieni w biologii. PWN, W-wa, 97–121 (in Polish).
25. Williams R.W., Dunker A.K., Determination of the structure of proteins from the Amide I band of the laser Raman Spectrum. J. Mol. Biol., 1981, 152, 783–813.
26. Williams R. W., Estimation of protein secondary structure from the laser Raman Amide I spectrum. J. Mol. Biol., 1983, 166, 581–603.
27. Wong P. T. T., Heremans K., Pressure effects on protein secondary structure and hydrogen deuterium exchange in chymotrypsinogen: a Fourier transform infrared spectroscopic study. Bioch. Biophys. Acta, 1988, 956, 1–9.
28. Wong P. T. T., Saint Girons I., Guillou Y., Cohen G. N., Barzu O., Mantsch H. H., Pressure--induced changes in the secondary structure of the *Escherichia coli* methionine repressor protein. Biochim. Biophys. Acta, 1989, 996, 260–262.

Received May 1997. Revision received October 1997 and accepted November 1997.

ZASTOSOWANIE SPEKTROSKOPII RAMANA DO BADANIA WPŁYWU WYSOKICH CIŚNIEŃ NA DRUGORZĘDOWĄ STRUKTURĘ BIAŁEK FASOLI (*PHASEOLUS VULGARIS*) I GROCHU (*PISUM SATIVUM*)

Mirosława Klepacka, Krzysztof Bajdor[1], Artur Jeżewski[2]

Wydział Technologii Żywności, SGGW Warszawa, [1]Instytut Chemii Przemysłowej, Warszawa; [2]Instytut Chemii Organicznej Polskiej Akademii Nauk, Warszawa

Badano przy użyciu spektroskopii Ramana wpływ wysokich ciśnień na drugorzędową strukturę białek fasoli i grochu. Białka rozpuszczone w D_2O poddawano presuryzacji przy 300 i 600 MPa. Pod wpływem presuryzacji następowało obniżenie udziału α-helisy i β-struktury a zwiększenie udziału struktury nieuporządkowanej (tab. 1). Wysokie ciśnienie wywoływały większe zmiany w β-strukturze białek grochu niż w białkach fasoli. Analiza pasm przy 853 cm^{-1} i 828 cm^{-1}, pochodzących z rezonansu Fermiego, wykazała, że w białku fasoli tyrozyna jest ukrywana w łańcuchu polipeptydowym a w białku grochu eksponowana na zewnątrz (tab. 4). Widma Ramana białek fasoli poddawanych ciśnieniom w stanie stałym różniły się od danych uzyskanych z białek rozpuszczonych w D_2O (rys. 1). Presuryzacja białek fasoli w stanie stałym wywoływała mniejsze zmiany w strukturze drugorzędowej niż w białku presuryzowanym w D_2O (tab. 3).