www.foodandfeed.fins.uns.ac.rs
FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad
ISSN
2217-5369
(print version ceased in 2023)
2217-5660 (online)

UDK 639.515:[66.061.3:577.112
DOI: 10.5937/ffr0-53099

Original research paper

VALORISATION OF SHELL WASTE OF INVASIVE CRAYFISH FROM DANUBE RIVER (*FAXONIUS LIMOSUS)*: PROTEIN EXTRACTION AND CHARACTERIZATION

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Abstract: In order to deal with invasive crayfish (*Faxonius limosus)* impact on the native crayfish, as well as fish biodiversity in the Danube River, a possible solution would be to find and adopt mechanisms for its utilization for novel valuable product production. Apart from utilizing edible parts for novel food products, the shell waste can also be considered a source of valuable compounds. The complex structure of the shell mainly consists of three basic compounds: chitin, protein and minerals-mainly calcium carbonate.
In this study, shell waste proteins were extracted using three extraction methods. The first method involved using naturally present enzymes (proteases and lipases) in crayfish wastes to recover proteins through an autolysis process. To accelerate the process, UV radiation was used. The remaining two extraction methods were alkaline extraction of proteins, wherein one method alkaline extraction was applied directly to the shell waste, and the other method, alkaline extraction followed an acidic demineralization step of the shell waste. The obtained protein concentrates were analysed for yield, crude protein content, DPPH radical scavenging ability, amino acid content, and structure.
The results have shown that a similar percentage of protein content was obtained by all three methods: 67.20-68.03 %, but the extraction yield was considerably different. Alkaline deproteinization with or without the step of demineralization resulted in a 9.20-10.98 % yield, while UV radiation accelerated autolysis resulted in only a 3.41 % yield. Although protein extraction without using exogenous enzymes or chemicals is a very interesting approach, a drawback of this approach is the low process yield. FTIR spectroscopy revealed a secondary structure that was similar in all three concentrates, according to peak deconvolution, with the autolytic concentrate differing to a lesser extent, showing a slightly higher share of β-sheet structures. The DPPH assay revealed high antioxidant activity of the concentrates (72.00-88.06 %), probably originating from active peptides derived from proteins and residual carotenoids, mainly astaxanthin.

Key words: *crayfish, shell waste, protein, amino acid, FTIR, DPPH*

INTRODUCTION

As a response to the situation of native fresh-water crayfish number drop, the spiny-cheek crayfish (*Faxonius limosus*) was deliberately introduced for use in aquaculture, first in Germany 1890s and then in Poland and France. Recently, records of the spiny-cheek crayfish have been reported from 22 different European countries. Its influence on indigenous species

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was described as devastating, due to the sum of factors, absence of natural enemies, feeding on aquatic vegetation, fish eggs and invertebrates, carrier of the crayfish plague, a highly infectious disease of all crayfish of non-North American origin, superior reproductive performance, tolerance to environmental conditions, and resistance to diseases (Śmietana, Panicz, Sobczak, Nędzarek, & Śmietana, 2020; Śmietana, Panicz, Sobczak, Śmietana, & Nędzarek, 2021; Roljić et al., 2024).

In Serbia, *F. limosus* was first reported in 2002 in the Danube near Apatin, and since then, it has spread along the entire section of the Danube River in Serbia, Sava, Tisa, Velika Morava, and Tamiš. It has been estimated that during the next few decades, allochthonous crayfish species will completely dominate indigenous populations (Zorić, Atanacković, Ilić, Csányi & Paunović, 2020; Roljić et al., 2024).

Although the knowledge of the invasive crayfish population continues to improve, efforts to stop or slow their growth have shown limited success. Apart from the "ark-sites" approach (areas where invasions are unlikely to reach in the existing conditions or with minor intervenetions), harvesting invasive species that may be a source of edible and non-edible raw material was proposed (Satmari et al., 2023; Śmietana et al., 2021).

Invasive crayfish *F. limosus* from the Danube River was tested as a new generation of food in terms of safety, technological quality, nutritive properties and sensory value of spiny-cheek crayfish meat to assess its potential usefulness in the food and feed industry (Lazarević et al., 2022; Śmietana et al., 2021). Considering that *F. limosus* is an invasive species, it could be expected that in the future adequate quantities of this raw material will be available (Zorić et al., 2020).

To the best of our knowledge, analysis of *F. limosus* shell waste protein structures was not published. This paper aimed to analyse the content, primary and secondary structure, as well as bioactivity of the protein fraction of the shell waste to open new protein valorisation directions, among which biopolymer materials could be an important field.

MATERIALS AND METHODS

Ground crayfish shell waste (granulation <0.5 mm) was obtained from the specimens of *F. limosus* captured in standard cages along the main course of the Danube river flowing through Begeč, Serbia, using a sampling procedure described in previous work (Lazarević et al., 2022). Crayfish shell waste was obtained after extracting the edible contents at the Institute of Food Technology, University of Novi Sad. The shell waste was boiled for 10 min in water, dried in the air, and then oven-dried at 55-65 °C. After drying, the shell waste was ground and sieved through appropriate sieves (<0.5 mm). All chemicals used were of p.a. quality.

Protein extraction from crayfish shell waste

Crayfish shell waste powder was used for protein extraction using three different extraction procedures. The first procedure (DP) involved alkaline extraction with 0.3N NaOH (Lach-Ner, Czech Republic) at a mass:volume ratio of 1:10 during 60 min at the temperature 80-85 °C. The second procedure (DMDP) included demineralization step before deproteinization. Demineralisation was performed with 0.5N HCl (Centrohem, Serbia) during 45 min at the room temperature, followed by filtration, washing to neutral pH, and drying. Deproteinization was then carried out as described in the first procedure (Rhazi, Desbrières, Tolaimate, Alagui & Vottero, 2000; Nadarajah, 2005; Ribeiro, Viana, Hattori, Constantino & Perotti, 2018). The third procedure (A) was performed using autolysis for protein extraction (Cao, Tan, Zhan, Li & Zhang, 2014). To intensify the autolysis process, UV irradiation (253.7 nm; 20 W; 20 min) was applied to a shell waste powder-water homogenate (1:1 w/v), followed with gradient temperature treatment where a 20 % (w/v) shell waste powder water suspension (pH 9) was subjected to autolysis with the temperature gradually increasing from 40 °C to 65 °C, at a rate of 5 °C per hour. In order to stop the reaction, after 7 hours, sample was heated to 100 °C for 10 min and cooled to room temperature.

After protein extraction, the reaction mixture was filtrated and filtrate was used to precipitate proteins by lowering the pH to 4.9. The precipitated proteins were left in the refrigerator overnight, followed by centrifugation at 4500 rpm for 20 minutes. The supernatant was discarded, and the precipitate was dried and used for further analysis.

Protein concentrate yield

The yield of protein concentrate, or simply yield, was determined by the ratio of the protein concentrate to the shell waste mass, as described by Abreu, De Souza, Da Rocha, Wasielesky and Prentice, (2019).

$$\text{Yield (\%)} = \frac{\text{Protein concentrate mass}}{\text{Shell waste mass}} \times 100$$

Protein content

The protein content in the obtained precipitates (concentrate) was determined using Kjeldahl method AOAC Official method 992.23 (AOAC, 1998). The conversion factor used was 6.25.

Determination of amino acid composition

Amino acids analyses of protein concentrates were performed by ion exchange chromatography using an Automatic Amino Acid Analyzer Biochrom 30+ (Biochrom, Cambridge, UK), according to method of Spackman, Stein & Moore (1958). The sodium acelerated buffer system consisted of four buffers with varying pH and a sodium hydroxide regeneration solution, with flow rates of 35 mL/hr. The technique was based on amino acid separation using strong cation exchange chromatography, followed by ninhydrin colour reaction and photometric detection at 570 nm, except for proline, which was detected at 440 nm. Samples were previously hydrolysed in 6M HCl (Merck, Germany) at 110 °C for 24 h. After hydrolysis, the samples were cooled to room temperature and dissolved in 25 mL of loading buffer (pH 2.2) (Biochrom, Cambridge, UK). Subsequently, prepared samples were filtered through 0.22 μm pore size PTFE filter (Plano, Texas, USA), and the filtrate was transferred into a vial (Agilent Technologies, USA) and stored in a refrigerator prior to analysis. Amino acid peaks were identified by comparison of retention times with retention times of amino acid standard purchased from Sigma Aldrich (Amino Acid Standard Solution (Sigma-Aldrich, St. Louis, USA). The results were expressed as the mass of amino acid (g) per 100 g of sample (Tomičić et al., 2022).

Fourier-Transform Infrared Spectroscopy (FTIR)

Structural properties of protein concentrates were determined using a Nicolet IS10 FT-IR spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) using attenuation total reflection (ATR) technique. Analysed spectra were recorded in the spectral range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}, taking 16 scans per record. Before scanning each sample spectra, a background spectrum was collected. The collected FTIR spectra were processed using Omnic 8.1 software (Thermo Fisher Scientific, Waltham, MA, USA).

For the purpose of analysing structural changes in proteins during formation of cast protein film, a film from DMDP concentrate was casted. The film-forming solution, composed of 10% of protein concentrate in water with addition of 30% glycerol as a plastificator (calculated to the mass of concentrate) was adjusted to pH 12 and subjected to 90 °C during 20 min in a water bath. After treatment, the solution was cast onto a Petri dish and dried under room conditions.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The assay was performed according to Šuput et al. (2024). Protein concentrate samples, as well as samples of the DMDP film, each approximately 0,03g, were incubated in the dark for 24 h at room temperature in a freshly prepared $DPPH^{\bullet}$ solution (0.0185 g $DPPH^{\bullet}$ in 50 mL ethanol), diluted in ethanol (1:5), and stirred during this period. Residual $DPPH^{\bullet}$ concentrations after 24 h of incubation were determined by measuring the absorbance at 520 nm using a T80/T80+ UV-VIS spectrophotometer (PG Instruments Ltd., Lutterworth, UK). The antioxidant activity of the films was expressed as a percentage and calculated using the following formula:

$$\text{AO (\%)} = \frac{((DPPH^{\bullet}_0 - DPPH^{\bullet}_s))}{(DPPH^{\bullet}_0)} \times 100$$

where $DPPH^{\bullet}_s$ is the concentration of $DPPH^{\bullet}$ in the tested film sample, and $DPPH^{\bullet}_0$ is the concentration of $DPPH^{\bullet}$ in the blank sample (without protein concentrate). For each film, the test was repeated three times, and the result was presented as mean ± SD.

Statistical analysis

Statistical analysis was conducted using OriginPro 8 (OriginLab Corporation, Northampton, MA, USA). Data were presented as mean value with their standard deviation indicated (mean ± SD). Variance analysis (ANOVA) was performed with a confidence

interval of 95% ($p < 0.05$). Means were compared using the Tukey's test.

RESULTS AND DISCUSSION

Protein content

Different methods of protein fraction extraction: DP, DMDP and A led to obtaining protein concentrate that had a dark brown colour and had 67.20-68.03 % of protein (table 1). Although a similar percentage of protein content was obtained in different concentrates, protein extraction yield was considerably different. Alkaline deproteinization with or without demineralization resulted in an approximate yield of 10%, while autolysis resulted in a significantly lower yield of 3.41%. Although the extraction of proteins without using exogenous enzymes or chemicals is an interesting approach, its major drawback is the low process yield.

The obtained results are consistent with data reported for protein content in red swamp freshwater crayfish (*Procambarus clarkii*) (Wang et al., 2022). They reported protein content of 4.85-10.98%, depending on crayfish weight, sampling month, and part of the shell. Similar results were reported for *Procambarus clarkii* (15.30%) by a different group of authors (El-Sherif, Abou-Taleb, Ibrahim, Talab & El-Ghafour, 2021).

Amino acid composition

The basic composition of the crustacean shell is formed of chitin nanofibers coated with proteins, with deposited calcium carbonate. Proteins in the crayfish shell structure include cuticular proteins (a broad class of proteins that form part of the chitin-protein matrix in the exoskeleton), chitin-binding proteins (which bind to chitin and strengthen the exoskeleton by linking the polysaccharide chains in a structured manner), mineral-associated proteins (which contribute to the calcification and overall hardness of the shell), and crustacyanin (a pigment-binding protein involved in the colouration of the exoskeleton, binding to astaxanthin) (Stirn, 2012).

The amino acid composition of protein concentrates is presented in Table 2. The total amino acid content amounts to 86.5-93.6% of the crude protein content (table 1), or somewhat lower due to the amino acid hydrolysis (Feng, Gao, Burgher, Zhou & Pramuk, 2016).

Proteins contributing to the rigidity of the shell are rich in hydrophobic amino acids (34.64-38.36%), while hydrophilic amino acids facilitate interactions with chitin and other components (36.87-38.80%). The high abundance of aspartic acid and glutamic acid could be related to the presence of acidic proteins, i.e. Ca^{2+} binding proteins (Endo, Takagi, Ozaki, Kogure & Watanabe, 2004). Cystine, which plays a key role in forming disulfide bonds that strengthen the exoskeleton, was detected only in the A concentrate. Also, amino acids involved in the chitosan-binding sequence of chitosan-binding proteins were detected in the analysed concentrates (Rebers-Riddiford consensus sequence, Gx8Gx6YxAxExGYx7Px2P, where x indicates any amino acid residue) (Suzuki, Sugisaka-Nobayashi, Kogure & Nagasawa, 2013). The results align with literature data on crustacean shell protein amino acid content. The most abundant non-essential amino acids in this study were glutamic and aspartic acid, consistent with reports for crab shells (*Sudanonaonautes aubryi*), freshwater crayfish *(Procambarus clarkii)* meal, as well as dried crayfish shells (*P. clarkii)*. For essential amino acids content, leucine and lysine were the most dominant in this study, while valine and arginine were the most prominent in crab shells (also present in significant amounts in this study), leucine and valine in freshwater crayfish meal *(P. clarkii)*, and lysine and tyrosine in freshwater

Table 1. Protein concentrate yield from crayfish shell waste (%), protein concentrate protein content (%) and DPPH radical scavenging activity (%)

Parameter	DP	DMDP	A
Protein concentrate yield (%)	9.20 ± 2.02^{a}	10.98 ± 0.21^{a}	3.41 ± 0.50^{b}
Protein concentrate protein content (%)	67.20 ± 6.08^{a}	67.37 ± 6.11^{a}	68.03 ± 6.18^{a}
DPPH activity (%)	82.45 ± 6.17^{ab}	72.00 ± 4.93^{b}	88.06 ± 1.97^{a}

DP-alkaline deproteinization of shell waste; DMDP-demineralization followed by deproteinization of shell waste; A-autolysis of shell waste; Different letters ab within the same row mark significantly different means with 95 % probability ($p < 0.05$)

Table 2.
Amino acids (TAA) composition of the tested sample, presented in g/100g of sample

Amino acids (AA)		A	DP	DMDP
Aspartic acid		7.83 ± 0.05	6.75 ± 0.04	7.68 ± 0.05
Threonine		3.22 ± 0.04	2.55 ± 0.03	2.23 ± 0.02
Serine		3.52 ± 0.03	2.14 ± 0.02	2.01 ± 0.01
Glutamic acid		8.96 ± 0.05	8.08 ± 0.05	8.90 ± 0.06
Proline		2.10 ± 0.02	1.90 ± 0.01	1.81 ± 0.01
Glycine		3.24 ± 0.03	2.85 ± 0.03	3.06 ± 0.04
Alanine		3.67 ± 0.03	3.19 ± 0.04	3.43 ± 0.03
Cysteine		0.77 ± 0.01	n.d.	n.d.
Valine		3.77 ± 0.04	3.62 ± 0.04	3.86 ± 0.03
Methionine		2.10 ± 0.02	2.55 ± 0.03	2.60 ± 0.02
Isoleucine		3.17 ± 0.03	3.28 ± 0.03	3.37 ± 0.03
Leucine		4.76 ± 0.04	5.00 ± 0.05	5.52 ± 0.04
Tyrosine		2.81 ± 0.02	3.12 ± 0.03	3.12 ± 0.03
Phenylalanine		3.82 ± 0.04	4.37 ± 0.04	4.44 ± 0.04
Histidine		2.01 ± 0.02	2.12 ± 0.02	2.10 ± 0.01
Lysine		4.54 ± 0.05	3.62 ± 0.04	3.52 ± 0.04
Arginine		3.38 ± 0.03	2.98 ± 0.03	2.89 ± 0.03
Total amino acids (TAA)		63.68 ± 0.55	58.13 ± 0.53	60.53 ± 0.49
Total essential amino acids		27.39 ± 0.28	27.11 ± 0.28	27.64 ± 0.23
Total non-essential amino acids		36.28 ± 0.27	31.01 ± 0.25	32.90 ± 0.26
AA with charged side chains (%)	Acidic side chains	26.37 ± 0.16	25.51 ± 0.15	27.39 ± 0.18
	Basic side chains	15.59 ±0.16	15.00 ± 0.15	14.06 ± 0.13
AA with uncharged side chains (%)	Polar side chains	15.00 ± 0.14	13.44 ± 0.14	12.16 ± 0.10
	Non-polar side chains	43.03 ± 0.41	46.03 ± 0.46	46.41 ± 0.40
Hydrophilic (%)		38.80 ±0.28	36.87 ± 0.28	37.98 ± 0.30
Hydrophobic (%)		34.64 ±0.33	37.86 ± 0.40	38.36 ± 0.31
Neutral (%)		26.54 ± 0.25	25.25 ± 0.24	23.67 ± 0.20

n.d. – not detected; A-autolysis of shell waste; DP-alkaline deproteinization of shell waste; DMDP-demineralization followed by deproteinization of shell waste

crayfish shell *(P. clarkii)* (El-Sherif et al., 2021; Ayodeji Ahmed, 2022; Makkey et al., 2023).

The side chains of acetic acids provide various possibilities for further applications. It has been reported that, as an intrinsic factor influencing protein solubility, aspartic acid, glutamic acid, and serine, on the surface of a folded protein contribute significantly more favourably than the other hydrophilic amino acids, especially at a high net charge (Trevino, Scholtz, & Pace, 2007).

Also, the high content of aspartic acid leaves the possibility of metal coordination, ionic bonds (salt bridges) could form between positively charged (basic) and negatively charged (acidic) amino acids, particularly in environments where pH permits charged interactions.

Hydrophobic residues may also contribute to the association of proteins with lipids or other hydrophobic surfaces, while various polar groups allow a broad spectrum of hydrogen bonding.

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectra were used to analyse the secondary structure of proteins in protein concentrates by deconvoluting amid I band (1700-1600 cm^{-1}) that mainly originates from carbonyl group of peptide bond stretching vibration and shows high sensitivity to the protein's secondary structure due to the involvement of the C=O group in hydrogen bonding and its environment (Bradley, 2007; Sukumaran, 2022; Wang et al., 2022).

The shape of the amide I peaks for all tested samples is shown in Fig. 1. A similar broad peak, shifted to lower wave numbers corresponding to a less ordered structure, can be observed. The amide I peaks of DP and DMDP concentrates are very similar, whereas in the A protein concentrate, an additional shift towards β-sheet structures (1620 cm^{-1}) can be observed. In contrast, the DMDP film exhibits a shift in the opposite direction. After the peak resolve of the amide I band, protein secondary structures were obtained, as shown in Fig. 2.

Protein secondary structure resolve suggested that the most rigid structure, α-helix was present at 23.0% in A, 23.6% in protein concentrates DP and DMDP and 24.9% in film. The less ordered structure of β-sheet was represented in the range 35.2% (DP and DMDP) -35.6% (A) in concentrates and 28.8% in film. Denaturised random coils and turns were represented at 41.1 % (DP and DMDP)-41.4 % (A) in concentrates and 46.3 % in film. The secondary structure was similar in all three concentrates, according to the peak deconvolution, with A concentrate differing to a lesser extent. In protein film, the share of β-sheet structure was decreased towards a more flexible random structure. This shift probably originates from the film synthesis procedure implying treatment pH12/90 °C/20 min.

The second peak related to the amide bond, which can complement information about present hydrogen bonds and secondary structures, is amide II (3000-3600 cm^{-1}).

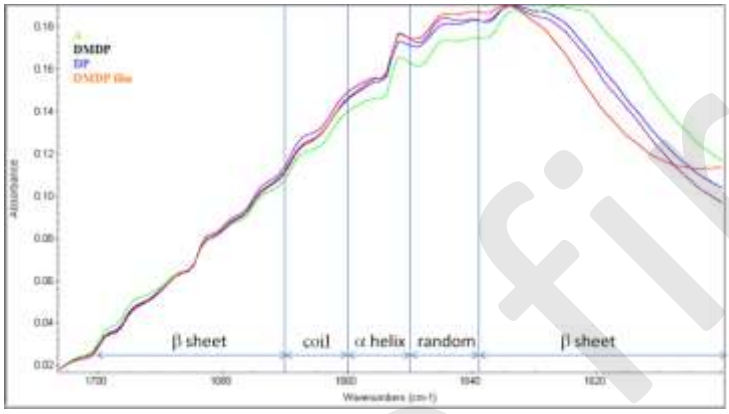

Figure 1. Shape of amide I peaks for all tested samples of protein concentrates: A-autolysis of shell waste; DMDP-demineralization followed by deproteinization of shell waste, concentrate and film; DP-alkaline deproteinization of shell waste

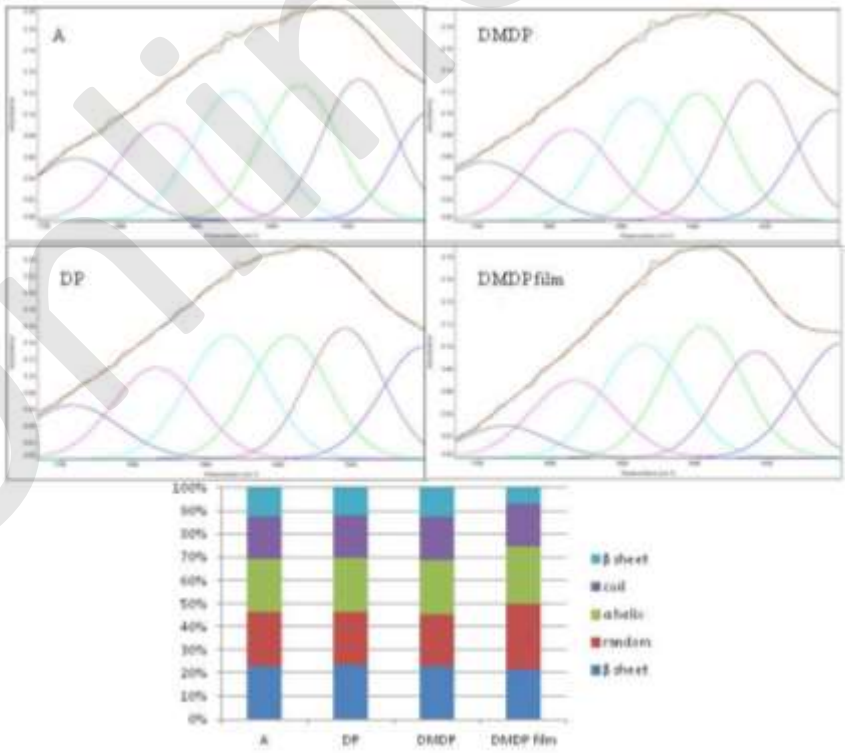

Figure 2. Peak resolve of the amide I band and protein secondary structures of tested samples DP-alkaline deproteinization of shell waste; DMDP-demineralization followed by deproteinization of shell waste, concentrate and film; A-autolysis of shell waste

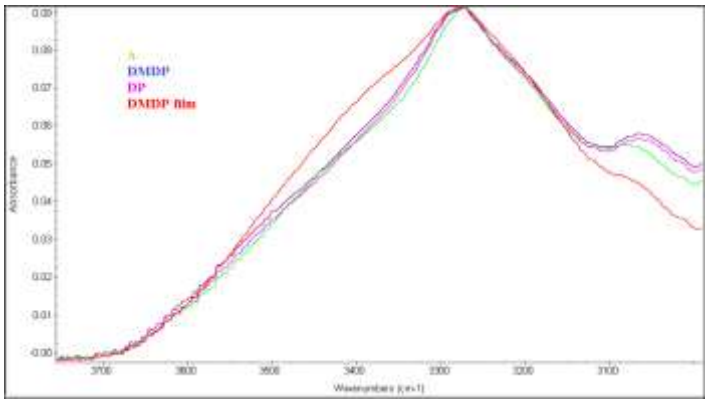

Figure 3. Shape of amide I peaks for all tested samples of protein concentrates: A-autolysis of shell waste; DMDP-demineralization followed by deproteinization of shell waste, concentrate and film; DP-alkaline deproteinization of shell waste

This broad peak is partly composed of overlapped peaks originating from the second part of the peptide bond (amide A and amide B bands). Amide A band (around 3300 cm^{-1}) corresponds to the NH stretching vibration in the peptide bond and a similar origin is of the amide B band (around 3100 cm^{-1}), although this band is usually weaker. As for the rest of the amide II wide peak, the broad peak from 3600-3200 cm^{-1} is attributed to the –OH groups of the amino acid side chains stretching vibrations, as well as water bonded to the protein molecules. Additional several peaks appear in the range 3000-3400 cm^{-1}, due to the primary and secondary amines in the protein backbone and side chains. Some CH stretching vibrations can overlap slightly into the 3000 cm^{-1} region, but they are more common around 3000-2800 cm^{-1} (Wang et al., 2022). Shape of this peak is in accordance with previous secondary structure analysis using amide I band, as shown in Fig. 3.

As already described in the amide I band peak deconvolution, two concentrates DMDP and DP are very similar. Differences can be observed for concentrate A, where the amide B bond (around 3100 cm^{-1}) is weaker, which is in accordance with the amide I shape and higher prevalence of β-sheet structure. Also, the amide II band of DMDP film shows an additional decrease of the amide B band, as well as broadening of the amide A band which is in accordance with the results from amide I peak deconvolution and higher representation of random structures. As already described in the amide I band peak deconvolution, two concentrates DMDP and DP are very similar. Differences can be observed for concentrate A, where the amide B bond (around 3100 cm^{-1}) is weaker, which is in accordance with the amide I shape and higher prevalence of β–sheet structure. Also, the amide II band of DMDP film shows an additional decrease of the amide B band, as well as broadening of the amide A band which is in accordance with the results from amide I peak deconvolution and higher representation of random structures.

DPPH assay

The results of the DPPH assay are presented in Table 1. All three samples demonstrated significant radical scavenging activity, in the range of 72.00-88.06%. These results can be compared to the results of substrates regarded as rich in antioxidants under similar testing conditions, such as *Camelina sativa* oilcake-based composite films, which exhibited DPPH scavenge ability of 78.04% and soybean sprouts, with scavenging activity ranging from 79 to 87.1% (Mugisha, Asekova, Kulkarni, Park & Lee, 2016; Šuput et al., 2024).

Obtained high values for radical scavenge activity could originate from two basic source groups, where the first includes active peptides derived from proteins, as well as specific amino acids, such as cysteine, tyrosine, and histidine, known for their antioxidant properties which may contribute to the DPPH scavenging activity (Dey & Dora, 2014; Li et al., 2020; Dragojlović et al., 2021; Zhou, Dai, Huang, & Qin, 2021). The second group consists of residues of highly potent active compounds from the shell, particularly carotenoids, with astaxanthin being the most prominent. Although carotenoids are more stable when esterified, it was also reported that they interact with proteins (carotenoproteins) or lipoproteins (carotenolipoproteins) (Ahmadkela-

yeh & Hawboldt, 2020; Šimat, V., Rathod, N. B., Čagalj, M., Hamed, I., & Mekinić, 2022). Comparable DPPH radical scavenging activity, similar to the values obtained in this paper, was reported for carotenoprotein extracted from shrimp *P. stylifera* (72.96%) (Pattanaik et al., 2020).

CONCLUSIONS

By alkaline deproteinization of the shell waste of the crayfish *Faxonius limosus* from the Danube, with and without the demineralization process, a similar yield (9.20-10.98%) of protein concentrate with a protein content of 67.20-67.37% was obtained. Using UV-accelerated autolysis without adding any chemicals, a concentrate with comparable protein content (68.03%) was produced, although the yield of the concentrate was significantly lower. With minor differences in the composition of the individual amino acids, the most common non-essential amino acids in all examined protein concentrates were glutamic acid and aspartic acid, and the essential amino acids were leucine and lysine. The secondary structure of the proteins (according to the analysis of the amide I and amide II bands of the FTIR spectra) was similar in all three concentrates; with the A concentrate deviating to a lesser extent. In the protein film, the proportion of β-sheet structure decreased in favour of a more flexible random structure, probably due to the film synthesis procedure. The results of the DPPH assay for all three samples of the protein concentrates showed significant free radical scavenging activity of 72.00-88.06%, which probably originated from active peptides and amino acids as well as from residues of carotenoids, led by astaxanthin.

AUTHOR CONTRIBUTIONS

Conceptualization, N.M.H. and I.S.Č.; Methodology, N.M.H and J.R.P.; Investigation, formal analysis, validation, N.M.H., S.M.R., Z.M.T.; Writing-original draft preparation, N.M.H.; Writing-review and editing, N.M.H., D.Z.Š.; Supervision. S.Z.P

DATA AVAILABILITY STATEMENT

Data contained within the article.

ACKNOWLEDGEMENTS

The research was supported by the Science Fund of the Republic of Serbia, 7417, Reducing the negative impact of invasive crayfish *Faxonius limosus* in the Danube by smart exploitation of their meat and shells – DANUBEcare, as well as the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Registration numbers: 451-03-66/2024-03/ 200134 and 451-03-65/2024-03/ 200134).

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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VALORIZACIJA LJUŠTURE INVAZIVNIH RAKOVA IZ REKE DUNAV (*FAXONIUS LIMOSUS*): EKSTRAKCIJA I KARAKTERIZACIJA PROTEINA

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Sažetak: U cilju regulisanja uticaja invazivnih rakova *Faxonius limosus* na autohtone rakove, kao i biodiverzitet ribe u reci Dunav, moguće rešenje bi bilo pronalaženje i usvajanje mehanizama za upotrebu ovog raka za proizvodnju novih vrednih proizvoda. Pored upotrebe jestivog dela za nove prehrambene proizvode, ljuštura se takođe može smatrati izvorom vrednih jedinjenja. Kompleksna struktura ljušture uglavnom se sastoji od tri osnovna jedinjenja: hitina, proteina i minerala-uglavnom kalcijum karbonata.
U ovom radu, proteini ljušture su ekstrahovani korišćenjem tri metode ekstrakcije. Prvi metod je bio korišćenje prirodno prisutnih enzima (proteaza i lipaza) u otpadu od rakova i izdvajanje proteina korišćenjem procesa autolize. Da bi se ubrzao proces, korišćeno je UV zračenje. Preostale dve metode ekstrakcije bile su alkalna ekstrakcija proteina, pri čemu je u jednoj metodi alkalna ekstrakcija primenjena direktno na ljušturu, a u drugoj metodi alkalna ekstrakcija prati korak kisele demineralizacije ljušture. U dobijenim koncentratima proteina analizirani su: prinos, sadržaj sirovog proteina, sposobnost uklanjanja radikala DPPH, sadržaj aminokiselina i struktura.
Rezultati su pokazali da je sličan procenat sadržaja proteina dobijen nakon sve tri metode ekstrakcije: 67-68%, ali je prinos ekstrakcije bio znatno drugačiji. Alkalna deproteinizacija sa ili bez koraka demineralizacije dala je prinos od oko 10%, dok je autoliza ubrzana UV zračenjem dala samo 3,41% prinosa. Iako je ekstrakcija proteina bez upotrebe egzogenih enzima ili hemikalija veoma interesantan pristup, nedostatak ovog pristupa je nizak prinos procesa. FTIR spektroskopijom je određena sekundarna struktura koja je bila slična u sva tri koncentrata, prema dekonvoluciji pikova, pri čemu se autolitički koncentrat razlikovao u manjoj meri, zbog većeg udela strukture β ravni. DPPH test je pokazao visoku antioksidativnu aktivnost koncentrata (72-88%), koja verovatno vodi poreklo od aktivnih peptida dobijenih iz proteina i ostataka karotenoida predvođenih astaksantinom.

Ključne reči: *rak, ljuštura, protein, amino kiselina, FTIR, DPPH*

Received: *30 August 2024/* ***Received in revised form:*** *19 September 2024/* ***Accepted:*** *23 September 2024*
Available online: *October 2024*

This work was reported at the FoodTech 2024 "Science Today, Technology Tomorrow" congress held in Novi Sad, Serbia, October 16-18, 2024 organized by the Institute of Food Technology in Novi Sad.