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Original research paper

ANTIOXIDATIVE AND FUNCTIONAL PROPERTIES OF PLUM OIL CAKE PROTEIN ISOLATE PREPARED BY DIFFERENT DRYING METHODS

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Abstract: The global demand for proteins is constantly increasing, resulting in the need for science and industry to explore novel raw materials for protein extraction. Plum oil cake, obtained after plum oil cold pressing, has great potential as a nutritious, low-cost material. The high protein content (up to 50%) of this by-product is ideal for valuable protein-rich ingredients extraction. Protein isolates from plum oil cake (PPI) were prepared using different drying methods- thermal drying (PPIT) and freeze-drying (PPIF). Obtained isolates were compared in terms of their antioxidative properties and techno-functional properties. Protein content and process yield were also examined, resulting in high protein content (over 96%) with no influence of the drying method. The light colour of PPIF would be more appealing to consumers and more suitable for incorporation into food systems. The functional properties of the protein isolates were not significantly affected by different drying methods except for protein solubility. Both PIs exhibited minimum protein solubility at pH 5.0 and maximum solubility at pH 10.0, while PPIF was much more soluble than PPIT. The freeze-drying method led to a much higher antioxidant activity of PPIF. Overall, protein isolates from plum cake obtained from different processing methods differed in appearance, solubility and antioxidant capacity, but yield, protein content and other functional properties were similar. This information will be useful in optimising the production of this protein isolate and benefitting its applications.

Key words: *plant protein, thermal drying, freeze drying, protein solubility, colour*

INTRODUCTION

Although animal-based proteins are leading in terms of their nutritional properties, the market for plant proteins is expanding to meet growing consumer demand. There are two main reasons why consumers worldwide are concerned about the use of animal proteins: health issues and environmental impact (higher greenhouse gas emissions linked to climate chan-

ge). Therefore, plant proteins are an alternative source of low-cost dietary proteins for direct consumption that can replace animal proteins. Concerning population growth, science and the food industry face the ongoing challenge of finding new sources of plant proteins. The popularization of the zero-waste concept in the food industry has shifted focus towards finding

ways to revalue and utilize various by-products. Protein-rich by-products from the agro-food sector can be classified into industrial and agricultural groups (Hadidi et al., 2024). Industrial residues include pomaces, peels, cakes, and wastewater from food processing technologies (Sadh, Duhan & Duhan, 2018). Agricultural residues involve components such as husks, seed pods, leaves, stems, roots, and others.

Protein-rich by-products were previously widely used as animal feed, but their use has been extended to food production (Bhatnagar et al., 2024), due to their excellent functional and nutritional properties thus contributing to a circular economy. Among the most represented protein-rich by-products in Serbia are oilseed cakes (sunflower, soybean, hemp seed, rapeseed, pumpkin seed, linseed, plum kernel, apricot kernel, etc.). The protein content in oilcake varies between 22.21% (hemp seeds) and 61.02% (hull-less pumpkin seeds) (Popović et al., 2020; Mirpoor et al., 2022).

Oilseed cakes have great potential for the production of various value-added protein-based products (Danyliv, Vasilenko, Ozherelyeva & Kutsova, 2024; Taghdiri, Emtiazjoo, Azizi, Ariaai & Sedaghati, 2024; Čakarević et al., 2021a). Protein isolates obtained from oilseed cake vary in their functional properties, e.g. emulsification, cream stability, water and oil holding capacity, but are shown to be very comparable to protein isolates derived from other raw materials (Meram & Tontul, 2024; Taarji et al., 2024).

The cake obtained after plum seeds cold-pressing has great potential due to its high protein content. Plum oil cake (POC) has a protein content of up to 50% (Čakarević et al., 2021b). Therefore plum protein isolate (PPI) is gaining increasing attention as an ingredient that can be used not only in isolation but also in food, cosmetic and pharmaceutical products (Sheikh et al., 2022b).

This protein isolate, in addition to having good functional properties (solubility, water and oil adsorption capacity and emulsifying capacity), has a potential for the production of bioactive peptides that have a positive influence on body functions, so that PPI can be considered as a useful agent for reducing degenerative diseases (González-García, Marina & García, 2014). In

addition, PPI has been used in biopolymer material synthesis (Sheikh, Saini & Sharma, 2023a), demonstrating its potential as a good matrix film carrier. Considering the importance and potential of PPI, the present study focussed on investigating the influence of different drying methods (thermal and freeze-drying) on the functional and antioxidant properties of the isolates obtained as well as on the protein yield.

MATERIALS AND METHODS

Materials

Plum oil cake was kindly provided from “All nut”, Belgrade, Serbia. The cake was kept in the freezer until further investigation. All chemicals employed for the study were bought from Sigma Aldrich chemicals, St. Louis, MO, USA.

Methods

Preparation of plum oil cake protein isolate (PPI)

Protein isolate from plum oil cake obtained by cold pressing was extracted by alkali extraction with isoelectric precipitation (Fig. 1.). Plum oil cake, double defatted with n-hexane in ration 1:5, was thoroughly blended with distilled water in a 1:15 ratio. The pH of the slurry was maintained at 10.5 (with 1M NaOH) and kept in a shaking water bath at 40 °C for 90 minutes.

After extraction the slurry was filtered using Bihner’s funnel to remove the insoluble material. Isoelectric precipitation with diluted acid (2M HCl) was used to precipitate the dissolved proteins by adjusting pH (4.5-5.0). After protein precipitation overnight in the fridge, supernatant was removed by vacuum.

In the first method, the precipitate was centrifuged for 15 minutes at 8.500 rpm and 4 °C (Eppendorf 5804R centrifuge-0536, Hamburg, Germany). The precipitate was dried at 40 °C for 12 hours and then ground to obtain the PI powder.

In the second method, the precipitate was directly frozen in an ultra-freezer (Arctiko ULUF 450 -86 °C Ultra Low Upright Freezer, UK). The frozen PI was dried for 72 hours in a Freeze-dryer (Alpha 2-4 LSCplus, Martin Christ, German) at -40 °C and 0.128 bar.

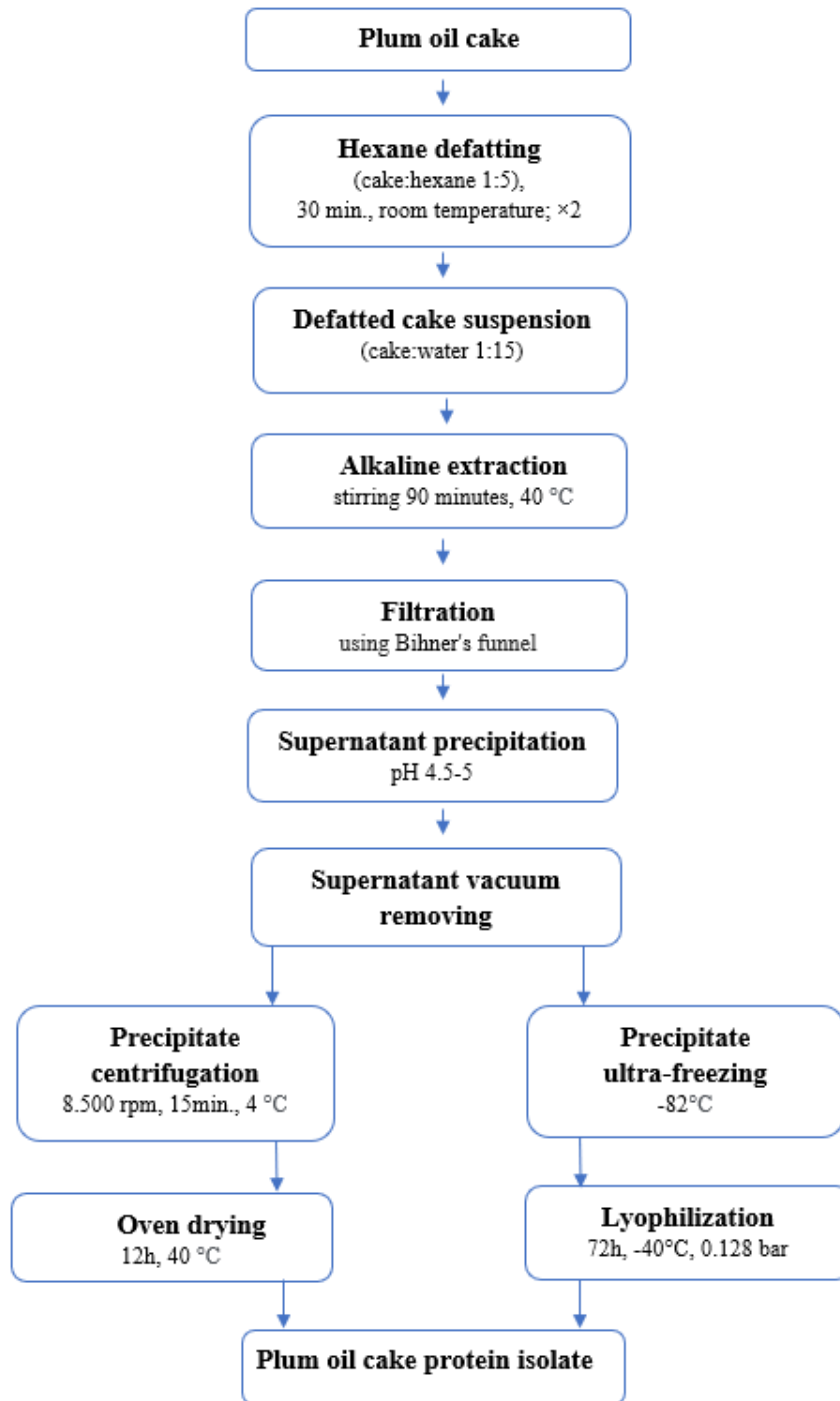


Figure 1. Schematic diagram of plum oil cake protein isolation using different drying methods

Protein content

Protein content of the protein isolates was analysed following the AOAC Official method 992.23 (AOAC, 1998). The determined nitrogen content was converted to protein concentration by multiplying it with the factor 6.25.

Colour measurements

Colour measurements were carried out in triplicates using a Chroma Meter CR-400 (Ko-

nica Minolta Co., Ltd., Osaka, Japan). Before the test, the instrument was calibrated using a standard light white reference tile and the measurements were performed under standard illuminant D65.

The results were expressed in terms of L* (lightness), a* (redness to greenness-positive to negative values, respectively), and b* (yellowness to blueness-positive to negative values, respectively) values.

Protein solubility

Protein isolate (PI) solubility was adjusted to pH 2.0–10. PI (10 mg) was weighed into Eppendorf tubes and added to 1 mL of a proper buffer solution. The prepared samples were constantly stirred for 1.5 h at 25 °C, using a Thermo-Shaker TS-100C (BioSan, Riga, Latvia). Soluble proteins from supernatant were determined according to Lowry, Rosenbrough, Fair and Randall (1951), using BSA (bovine serum albumin) as a standard. A series of standard solutions of BSA in the concentration range of 0.05–0.6 mg/mL was prepared. The absorbance was measured at 660 nm, and the protein content of the samples was calculated based on the standard curve obtained with BSA.

Water and oil absorption capacity

Method described by Rodsamran and Sothornvit (2018) with some modifications was used to determinate the water absorption capacity (WAC) and oil absorption capacity (OAC). Briefly, 100 mg of PI were mixed with 1 mL of distilled water or sunflower oil in a vortex for 1 min. After incubation at 30 °C for 30 min in Thermo-Shaker TS-100C (BioSan, Riga, Latvia), the tubes were centrifuged at 14.500 rpm for 20 min (Eppendorf Mini-spin plus, Eppendorf AG., Hamburg, Germany) at 25 °C. Water and oil were removed by inverting the tubes. The tubes with samples were weighed and WAC and OAC were calculated by the following equation Eq. (1) and Eq. (2):

$$WAC (g/g) = \frac{(W_2 - W_1)}{W_0} \quad \text{Eq. (1)}$$

$$FAC(g/g) = \frac{(F_2 - F_1)}{F_0} \quad \text{Eq. (2)}$$

Where W_2 , F_2 are weights of the tubes + the sediment; W_1 , F_1 are weights of tube + dry sample; W_0 , F_0 are weights of dry samples.

Foaming capacity and stability

The method described by Rodsamran and Sothornvit (2018) was slightly modified to determine the foaming capacity (FC) and foam stability (FS). Half a gram of PI was mixed with 50 mL of buffer (0.1 M sodium phosphate buffer pH 10.0) and then homogenised in an Ultra-Turrax T25 (Heidolph, Schwabach, Germany) at 10,000 rpm for 2 minutes. The volume was recorded before and after whipping. The whipped protein solution was transferred

to a 100 mL graduated cylinder and the volume was recorded at 0 min (V_0), 10 min, 20 min, 30 min, 60 min, 90 min and 120 min. Values for FC and FS were calculated according to the formula Eq. (3) and Eq. (4):

$$FC (\%) = \frac{(V_1 - V_2)}{V_2} * 100 \quad \text{Eq. (3)}$$

where V_1 , was the total volume after stirring, V_2 was the total volume before stirring.

$$FS (\%) = \frac{V_t}{V_k} * 100 \quad \text{Eq. (4)}$$

where V_t , was the volume of foam at time, V_k was the foam volume immediately after stirring.

Antioxidative activity - DPPH radical scavenging activity

The DPPH radical scavenging activity of plum oil cake protein isolate was evaluated according to the procedure reported by Popović et al. (2017). The absorbance values were measured at 520 nm (T80 UV-Vis Spectrophotometer; PG Instruments, Lutterworth, UK) and the DPPH radical scavenging activity was calculated by the following equation Eq. (5):

$$DPPH (\%) = \frac{(A_{blank} - A_{sample})}{A_{blank}} * 100 \quad \text{Eq. (5)}$$

where A_{blank} is the absorbance value at 520 nm of the ethanolic solution of DPPH without the added sample and A_{sample} is the absorbance value at 520 nm of the ethanolic solution of DPPH with the added sample.

Statistical analysis

Experimental data are presented as mean \pm standard deviation, and all experiments were performed at least in duplicate. The results were analysed by t-test, which was performed by XLSTAT (2022.1.2., Lumivero, Paris, France). The significance of differences among the mean values was indicated at the 95% confidence level.

RESULTS AND DISCUSSION

Yield and protein content

To evaluate the effects of various drying methods on the extraction of protein isolates, the protein content and yield were compared (Table 1). Different yields were obtained for PPIT and PPIF (20.80% and 19.62, respectively) (p-

value was 0.006). The yield is expressed as gross weight and a percentage ratio of the defatted cake. Regarding the low protein yield from plant-based raw materials, it was found that both PPIT and PPIF have a good yield.

High protein contents were observed in both PIs (over 96%), whereas protein content in PPIT was higher (p-value 0.003), which led to the conclusion that the drying technique influenced the protein content. Čakarević (2021b) found a higher protein content (up to 99%) in different plum cakes (after cold pressing and supercritical fluid extraction) than Hadnađev et al. (2018), who reported protein contents in PIs from hemp seed meal prepared by isoelectric precipitation and micellisation around 98% and 91%, respectively.

Colour attributes

The protein powders are shown in Fig. 2, followed by the CIE colour characteristics in Table 2. There were obvious differences between the protein powders - not only in colour but also in structure. PPIT was firmer (difficult to grind after drying), while PPIF was smooth and soft (there was no need for grinding).

PPIT was characterized by significantly lower lightness (higher L* value), and higher redness (a*) than the PPIF isolate (p-values were <0.0001). Yellowness (b*) was almost the

same (p-value was 0.051). Colour differences among the protein isolates can be attributed to the application of different drying techniques, probably because of alkaline conditions in combination with a higher temperature that can favour the co-extraction of phenolics (Vujetić et al., 2024; Hadnađev et al., 2018), resulting in the development of darker colour presented in PPIT.

Protein solubility

The functional properties influence the behaviour of proteins in food systems during processing, storage, preparation and consumption.

Solubility is one of the most important properties of proteins as it has a direct impact on the other functional properties. The solubility of both protein isolates as a function of pH (2.0 to 10.0) is shown in Fig. 3. Both PIs had a solubility curve with a characteristic U-shape.

For both PIs, the lowest solubility was achieved at pH 4.0 (16.22% and 30.43%, for PPIT and PPIF, respectively), while the highest solubility was at pH 10.0 (63.05% and 98.86% for PPIT and PPIF, respectively). Among PPIs, a much higher solubility was observed in PPIF, probably due to its structure, which proved to be smoother and softer compared to PPIT, which was difficult to grind.

Table 1.

Gross yield (%) and protein content (%) of plum oil cake protein isolates obtained by thermal (PPIT) and freeze-drying (PPIF)

| Isolation technique | Yield, based on defatted plum oil cake (%) | Protein content (% dry matter basis) |
|---------------------|--|--------------------------------------|
| PPIT | 20.80±0.12 | 98.21±0.15 |
| PPIF | 19.62±0.21 | 96.71±0.57 |



Figure 2. PIs obtained from plum oil cake by thermal (PPIT) and freeze-drying (PPIF)

Table 2.

CIE colour of PIs from plum oil cake obtained by thermal (PPIT) and freeze-drying (PPIF)

| Isolation technique | L* | a* | b* |
|---------------------|------------|-----------|------------|
| PPIT | 45.63±0.58 | 7.18±2.47 | 18.46±0.57 |
| PPIF | 61.82±9.26 | 2.70±0.57 | 18.19±0.15 |

Table 3.

Water absorption capacity and oil absorption capacity of PIs obtained from plum oil cake by thermal (PPIT) and freeze-drying (PPIF)

| Isolation technique | WAC (g/g PI) | OAC (g/g PI) |
|---------------------|--------------|--------------|
| PPIT | 2.16±0.02 | 1.23±1.43 |
| PPIF | 1.13±0.01 | 3.73±1.72 |

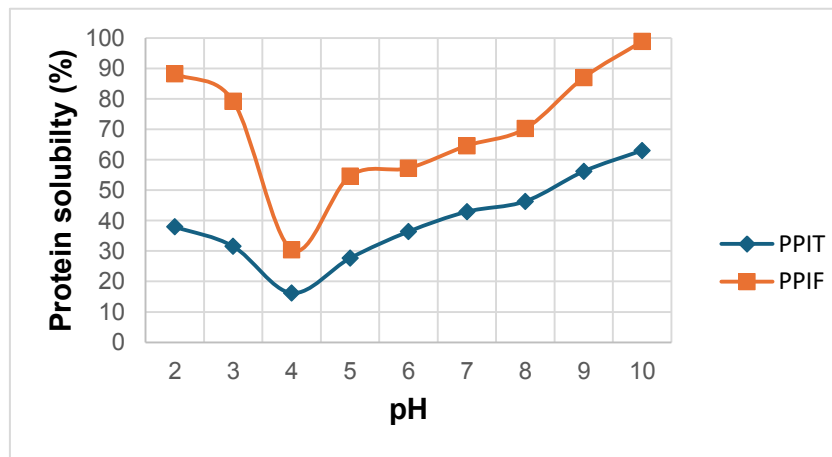


Figure 3. Influence of pH on protein solubility of PPIs obtained by thermal (PPIT) and freeze-drying (PPIF)

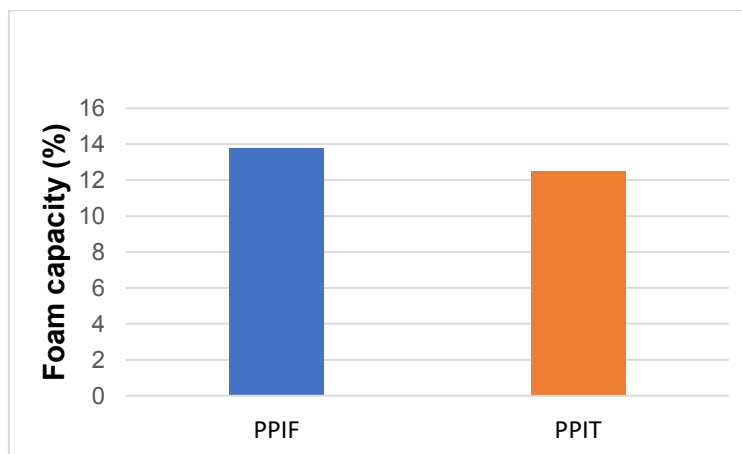


Figure 4. Foam capacity (%) of PPIs obtained by thermal (PPIT) and freeze-drying (PPIF)

According to results from this study, pI for extracted proteins was at pH = 4.0, which is in correlation with the general trend that most food proteins are acidic, with an isoelectric point at pH 4.0–5.0, but maximum solubility

was reached in alkaline conditions (at pH 10.0).

Maximal solubility was also obtained in alkaline conditions (at pH 8.0) in apricot kernel protein isolate (Čakarević et al., 2019a).

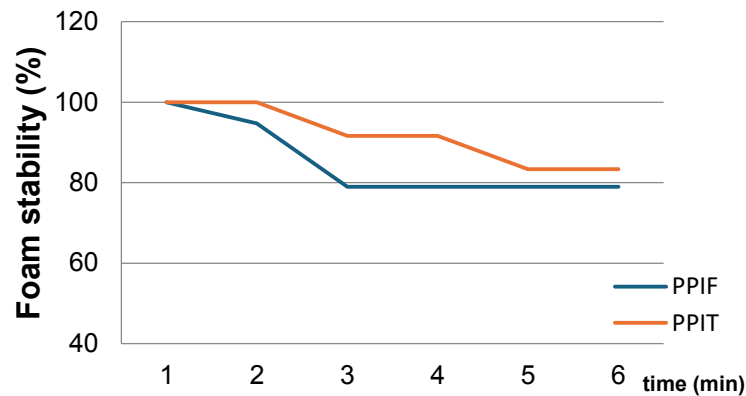


Figure 5. Foam stability (%) of PPIs obtained by thermal (PPIT) and freeze-drying (PPIF)

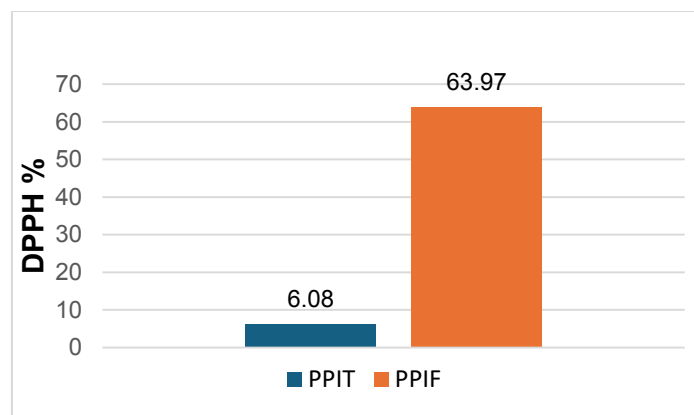


Figure 6. DPPH radical scavenging activity (%) of PPIs obtained by thermal (PPIT) and freeze-drying (PPIF)

Water and oil absorption capacity

WAC and OAC influence the texture and mouthfeel characteristics of foods and food products. The results of WAC and OAC are summarized in Table 3. The WAC and OAC statistically differed between the two PIs (p -values were 0.002 and 0.001, respectively). The present WAC value was higher in PPIT, whereas OAC was higher in PPIF, thus concluding that thermal drying had a positive influence on water absorption capacity while freeze-drying positively influenced oil absorption capacity.

The results for WAC and OAC for both PIs in this study were higher than the values reported for apricot protein isolate (Čakarević et al., 2019b) and similar to the values reported for hemp seed protein isolate (Hadnadev et al., 2018). Plum protein isolates obtained with supercritical carbon dioxide showed significantly improved water and oil absorption capacity (Sheikh, Saini & Sharma, 2023b), which

depends on amino acid composition, protein conformation, surface polarity and surface hydrophobicity (Saetae, Kleekayai, Suntornsuk & Jayasena, 2011).

Foaming capacity and stability

Figures 4 and 5 illustrate the foaming capacity and stability of the obtained protein isolates (PPIs). The foaming capacity (FC) values reached a maximum of 13.75%, with PPIF exhibiting a significantly higher foam capacity ($p = 0.006$). Both PPIs demonstrated good foam stability, although their foaming capacity was relatively low.

The current foaming capacity (FC) was lower than the values reported for peanut protein isolate and hemp seed protein isolate (Othmeni, Karoui & Blecker, 2024; Liu et al., 2023). The good foaming capacity of protein isolates is mainly attributed to their ability to form interfacial films to encapsulate bubbles thus contributing to their great potential for application as ingredients in food formulations.

Flexible and hydrophilic protein molecules are better foam stabilisers than more rigid molecules due to their ability to reduce surface tension (Herneke, Karkehabadi, Lu, Lendel & Langton, 2023).

Antioxidative activity

Since antioxidants have a beneficial effect on human health, there is a growing interest in finding novel raw materials for antioxidant extraction. Valuable sources are oilseeds, oil cakes, defatted seeds, etc. (Savić & Savić-Gajić, 2022). The antioxidative activity of plum seed was around 28 % (Sheikh et al., 2022a), while the antioxidative activity of plum oil cake obtained by cold pressing reached 70% (Ugarković et al., 2022).

The antioxidant potential of PIs obtained under distinctive drying conditions was estimated by using DPPH radical scavenging activity (Fig. 6).

The antioxidant potential of the PPI obtained by thermal drying showed a low activity (6.08%), while the PPI obtained by freeze-drying showed an activity of 63.97%. It can be concluded that freeze-drying had a positive effect on the antioxidant activity of the plum protein isolate. This activity could be attributed to polypeptides and/or other low molecular weight soluble ingredients contained in the plum oil cake (Čakarević et al., 2019b) which remain in a certain amount of liquid left after the supernatant vacuum removing step (Fig. 1.) during the process of protein isolation.

CONCLUSIONS

In this study, plum oil cake protein isolates were dried using different drying techniques - thermal drying and freeze-drying. The obtained isolates differed in appearance after drying. The plum protein isolate obtained by thermal drying (PPIT) was darker, firmer and rougher than the isolate treated by freeze-drying (PPIF). Protein content in both isolates was high- over 96%. The yield after both drying methods was about 20%. Freeze-drying resulted in a significantly higher solubility of the protein isolates, while the isoelectric point was the same for both isolates at pH 4. Given their comparable functional properties in water and oil holding capacity as well as foam formation, the two plum protein isolates are expected to be suitable for use in the same food systems. Notably, PPIF exhibited antioxidant activity

that was ten times higher, suggesting that non-thermal drying methods may be preferable for obtaining compounds capable of scavenging free radicals.

AUTHOR CONTRIBUTIONS

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

DATA AVAILABILITY STATEMENT

Data contained within the article.

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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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ANTIOKSIDATIVNA I FUNKCIONALNA SVOJSTVA PROTEINSKOG IZOLATA IZ ULJANE POGAČE ŠLJIVE DOBIJENOG RAZLIČITIM METODAMA SUŠENJA

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Sažetak: Globalna potražnja za proteinima je u stalnom porastu, što rezultira potrebom nauke i industrije da istražuje nove sirovine za ekstrakciju proteina. Uljana pogača šljive, dobijena hladnim ceđenjem ulja iz semena šljive, ima veliki potencijal kao hranljiv i jeftin sporedni proizvod. Visok sadržaj proteina (do 50%) čini ovaj nusproizvod idealnim za ekstrakciju vrednih sastojaka kao što su proteini. Proteinski izolati iz uljane pogače šljive (PPI) pripremljeni su različitim metodama sušenja – termičkim sušenjem (PPIT) i sušenjem zamrzavanjem (PPIF). Upoređena su antioksidativna i tehno-funkcionalna svojstva dobijenih izolata. Takođe, ispitan je sadržaj proteina i prinos ekstrakcije, koji je iznosio preko 96% u oba uzorka, bez uticaja metode sušenja. Svetla boja PPIF-a bi bila privlačnija za potrošače i pogodnija za inkorporaciju u sisteme hrane. Različite metode sušenja nisu značajno uticale na funkcionalna svojstva proteinskih izolata, osim na rastvorljivost proteina. Oba PI su pokazala minimalnu rastvorljivost na pH 5,0 i maksimalnu rastvorljivost na pH 10,0, dok je PPIF bio rastvorljiviji od PPIT. Sušenje zamrzavanjem dovelo je do mnogo veće antioksidativne aktivnosti PPIF-a. Sve u svemu, proteinski izolati iz uljane pogače šljive dobijeni različitim metodama sušenja razlikovali su se po izgledu, rastvorljivosti i antioksidativnom kapacitetu, ali su prinos, sadržaj proteina i druga funkcionalna svojstva bili slični. Ove informacije će biti korisne za dalju optimizaciju ovog proteinskog izolata i njegovu primenu u formulacijama hrane.

Ključne reči: *biljni protein, termičko sušenje, sušenje zamrzavanjem, rastvorljivost proteina, boja*

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