Determination of Physico-Chemical and Functional Properties of Plum Seed Cakes for Estimation of Their Further Industrial Applications

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Abstract: The extraction of bioactive compounds from the waste material in the food industry is an important approach because, in that way, the plant raw material can be utilized before its landfill disposal or combustion. The interest of scientists is great for the development of innovative procedures for the further application of these materials. Plum kernels obtained after plum processing can be used for the isolation of oil enriched with unsaturated fatty acids and cakes remaining after oil isolation from plant material. This study aimed to consider the possibilities of the further utilization of cakes obtained after oil isolation from plum seeds using organic solvents in the Soxhlet extractor. The physical–chemical and functional properties of the obtained cakes were determined. The results indicated that the plum seed cakes are rich in proteins (36.95–61.90%) and crude fiber (6.36–9.85%). The HPLC analysis showed that the highest content of phenolic compounds had coumaric acid in the concentration range of 11.31–12.98 mg/100 g of dry weight. The amygdalin content (0.005–0.139 mg/g of dry weight) was in the allowed concentration range so that the cakes can be considered safe for human use. The antioxidant potential of the cakes (IC₅₀ 0.40–0.65 mg/mL) indicated that antioxidants are also present in this waste material so that the cakes can be used as a raw material for the development of sustainable products in the food, pharmaceutical, and cosmetic industries.

Keywords: plum; seed cake; composition; functional properties; HPLC analysis; antioxidants

1. Introduction

World population growth and prolonging life expectancy cause reduced natural resources. To meet growing consumer demands and prevent environmental pollution, sustainable and efficient production processes are being developed. Food waste has the potential to be utilized in high-value-added products. Over the last few years, the valorization of food waste is one of the current areas of research that has attracted a lot of attention from many researchers. This approach is important because it is an alternative for the recovery of bioactive compounds from waste materials before their disposal in landfills [1]. There are a lot of species in the Prunus genus, but the number of economically important ones is quite low. Prunus domestica is commonly cultivated in Serbia compared to other Prunus species. Serbia is in third place in the world in annual plum production with about 430,000 t [2]. The plum fruit is used for the preparation of various food products (jam, compote, and marmalade, non-alcoholic and alcoholic beverages). The plum kernel represents about 10% of the total weight of plum fruit and is commonly used for pellets production as a sustainable energy alternative. In other words, this material is suitable for combustion and energy production [3]. In addition to this procedure, there are other ways of plum kernels utilization. After crushing the plum kernels, the seeds are used for oil production, as they contain a high amount of monounsaturated and polyunsaturated fatty acids [4]. The consumption of unsaturated fatty acids can cause many human health benefits, so their intake is suggestable on a daily basis [5]. The plum seeds contain about 30% of oil [6,7].
but after its isolation, the other 70% of its weight represents a cake that can contain many valuable bioactive compounds, such as protein [8], dietary fiber [9], carbohydrates, minerals, amygdalin [10], polyphenols [11], etc. In the literature, the different cakes of oilseeds (soybeans, oilseed rape, sunflower, pumpkin, coconut, cotton, palm, sesame, flax, etc.) are described [12]. The defatted cakes of various seeds have proteins, vitamins, carotenoids, minerals, polyunsaturated fatty acids, and tocopherols derived from residual oil as well as polyphenolic compounds [13]. Having in mind this composition, they can be used as a forage and in the development of innovative products. The plum seed cake represents a cheap source of protein that can be incorporated into food, cosmetic, and pharmaceutical products [14]. In our previous research, the oil from plum seeds was isolated using the solvents of different polarities in a Soxhlet extractor. The physico-chemical properties and oxidative stability of these oil samples were determined [6], while the cakes were not the subject of that study.

The aim of this study was to consider the potential and sustainable utilization of plum seed cakes. The cakes, representing the waste products after oil isolation using organic solvents, were subjected to further analysis for estimation of their physico-chemical and functional properties. The differences in the properties of plum seed cakes obtained using the solvent of different polarities were determined. Physico-chemical properties involved the determination of moisture content, crude fiber, and protein contents, as well as the pH value of the aqueous suspension of cakes. Amygdalin was quantified because its toxic products due to enzymatic hydrolysis limit the use of plum seed cakes [14]. Among the functional properties, the water absorption capacity, cake solubility index, oil absorption index, cake gelling ability, emulsifying, and foaming properties of cakes were determined. The presence of antioxidants was evaluated based on the antioxidant activity of cakes.

2. Materials and Methods
2.1. Chemicals and Reagents

Absolute ethanol, 99.5% (v/v) methanol, n-hexane, n-heptane, acetone, ethyl-acetate, chloroform, sodium carbonate, sodium hydroxide, sulfuric acid (Zorka Pharma, Sabac, Serbia), Folin–Ciocalteu’s reagent (AppliChem, Darmstadt, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol (HPLC grade), rutin, gallic acid, syringic acid, epicatechin, caffeic acid, and coumaric acid (Sigma Chemical, St. Louis, MO, USA) were used in this study.

2.2. Preparation of Plum Seed Cake

The dried plum seeds of Prunus domestica (Stanley European Plum) were purchased from Plemic doo (Osecina, Serbia). Before the treatment, the seeds were homogenized up to the particle size of 0.4 mm by grinding in the laboratory mill. The weighted seeds (100 g) were defatted by treating with 1 L of the organic solvents of different polarities in the Soxhlet extractor [8]. The obtained cakes were dried at a temperature of 45 °C in a laboratory oven for 24 h.

2.3. Physico-Chemical Properties

The moisture content in the analyzed plum seed cakes was determined using a standard procedure [15]. The cake samples (2 g) were dried at 105 °C in a laboratory oven for 2 h. After drying and cooling in the desiccator, the weight of the samples was measured. This procedure was repeated several times until a constant weight of the samples. The moisture content was calculated using Equation (1):

\[
\text{Moisture content (\%) } = \frac{w_0 - w_f}{w_o} \times 100
\]  

(1)

where \(w_0\)—the weight of the cake before drying (g), and \(w_f\)—the weight of the cake after drying (g).
The Kjeldahl and wet-burning methods were applied to determine the protein and crude fiber contents in the cakes, respectively [15]. The sample was first treated with a boiling solution of 1.25% sulfuric acid by heating for 1 h, which was then filtered and boiled in the solution of 1.25% NaOH for 1 h. After washing the samples, they were dried to a constant weight at 105 ± 10 °C.

The pH value of the plum seed cakes (1 g) suspended in 10 mL of distilled water was measured after being shaken at ambient conditions for 1 min [15]. The standard buffers pH 4.0 and 7.0 were used for the instrument calibration.

Amygdalin was quantified in the cakes according to the HPLC method [16]. The aqueous extract was prepared by the treatment of cakes (2 g) with 50 mL of water under reflux at 37 °C for 120 min. A Zorbax Eclipse XDB-C18 column (4.6 × 250 mm, 5 µm) enabled the separation of amygdalin from other present bioactive compounds in the samples. The content was presented as milligrams of amygdalin per gram of dry weight (d.w.).

2.4. Functional Properties

The investigation of the functional properties of cakes included the determination of water absorption capacity (WAC) according to the standard procedure [17,18]. The suspended cake (1 g) in 10 mL of distilled water was shaken at ambient conditions for 15 min. After that, the samples were centrifuged at 3500 rpm for 30 min. The WAC (g water/g of plum seed cake) was calculated based on the difference between the weight of the precipitate and the sample. The standard procedure [18,19] was used to determine the water solubility index (WSI) of cakes. The sample was prepared by suspending 2.0 g of cake in distilled water (10 mL). Hydrated cake samples were heated at 100 °C for 30 min and then cooled to ambient conditions. The dried supernatant was measured and presented per weight of the initial sample. To determine the oil absorption capacity (OAC), 0.5 g of milled plum seed cake was suspended in 10 mL of corn oil in a plastic centrifuge tube [17]. The suspension was stirred every 5 min at ambient conditions for 30 min. After that, the samples were transferred to plastic tubes and centrifuged at 4000 rpm for 15 min. The supernatants were decanted and dried, and the precipitated portion was measured. The OAC was expressed as the weight of bound oil in grams per 1 g of cake.

The aqueous suspensions of cakes were prepared in the concentration range of 2–10% to determine the lowest gelation concentration [20]. The samples were heated at 100 °C for 1 h and then cooled at 4 °C for 3 h. After inverting the tube, the suspension that did not fall or slip was considered the lowest gelation concentration.

The foam capacity (FC) and foam stability (FS) were determined according to the method of Lawhon et al. [21]. The suspension of cake (3 g/100 mL) in distilled water was adjusted to 7.0 and mixed at maximum speed for 5 min. After transferring the sample to a 250 mL measuring cylinder, the volume of foam was measured after 30 s. The contents were poured together with the foam into a 250 mL measuring cylinder, and the volume of the foam was measured after 30 s to define the FC (%) (Equation (2)) and FS (Equation (3)).

\[
\text{FC} (%) = \frac{\text{Volume after whipping} - \text{Volume before whipping}}{\text{Volume before whipping}} \times 100 \quad (2)
\]

\[
\text{FS} (%) = \frac{\text{Foam Volume after (t)time}}{\text{Initial foam volume}} \times 100 \quad (3)
\]

Emulsification activity (EA) of the aqueous suspension of cake (0.7 g/10 mL) was determined after adding and homogenization with the refined peanut oil (10 mL) in an electric blender for 5 min. The sample was centrifuged at 2000 rpm for 5 min, and the supernatant was left in the volumetric cylinder to separate the emulsified layers. The EA was calculated according to Equation (4):

\[
\text{EA} (%) = \frac{\text{Height of emulsified layer}}{\text{Height of total content in the tube}} \times 100 \quad (4)
\]
The analysis of emulsion stability (ES) was calculated according to Equation (5). The procedure was based on the centrifugation of the heated sample to 80 °C for 30 min and then cooling to 15 °C. After that, the emulsion was left to appear the layers for a few minutes.

\[
\text{ES} (\%) = \frac{\text{Height of emulsified layer after heating}}{\text{Height of total content in the tube}} \times 100
\]  

(5)

2.5. Antioxidant Potential of Plum Seed Cake

The ultrasound-assisted extraction of antioxidants from plum seed cakes was carried out under optimal conditions previously defined for non-fat plum seeds: the extraction time of 21 min, 21% (v/v) ethanol, the liquid-to-solid ratio of 28 mL/g, and extraction temperature of 66 °C [22]. The total antioxidant content (TAC) in milligrams of gallic acid equivalent per 100 g of dry weight (mg GAE/100 g d.w.) was determined for these extracts of cakes [23]. The scanning of the samples was carried out on a Varian Cary-100 spectrophotometer (Mulgrave, Victoria, Australia). The phenolic profile was confirmed using the HPLC method [24]. A Zorbax Eclipse XDB-C18 column (4.6 × 250 mm, 5 μm) (Agilent Technologies, Santa Clara, CA, USA) was used to separate the bioactive compounds.

2.6. Determination of the Antioxidant Activity

The DPPH assay was carried out to determine the antioxidant activity of the samples [25]. The half-maximal inhibitory concentration (IC\(_{50}\)) obtained by interpolation was used as a measure of antioxidant potential.

2.7. Statistical Analysis

The data are depicted as the mean of three measurements ± standard deviation. STATISTICA software 12 (Statsoft Inc., Tulsa, OK, USA) was used to carry out the statistical analysis. One way-analysis of variance was used to compare the mean value of obtained data. Tukey’s honestly significant difference (HSD) test as a post hoc test was applied to determine the statistical difference between the samples at the confidence level of 95% (\(p < 0.05\)).

3. Results and Discussion

3.1. Physico-Chemical Properties of Plum Seed Cake

The organic solvents were used for defatting the plum seeds. Despite the harmful effect on human health, they are still commercially used for oil production in the industry, because of their high extraction efficiency [26]. Soxhlet extraction was chosen as a suitable extraction technique since the satisfactory quality of oil and the constant chemical composition can be obtained compared to cold pressing. Although advanced extraction techniques are applied for the isolation of oil, some of them have not yet been used for commercial purposes due to techno-economic reasons. These techniques have significance for laboratory research but are still not for wide application. The basic quality parameters, the content of moisture, crude fiber, and protein, as well as the pH value of aqueous suspensions, were determined for plum seed cakes. The values of these parameters are shown in Table 1. Knowing the moisture content of the cake is important because it determines the storage conditions of the cake. The moisture level in the cake must be lower than the critical level, which for most seed cakes is below 10–12% [27]. The moisture content of the analyzed cakes ranged from 5.97% to 7.66%. The obtained values were lower than the critical value, which indicates the fact that the plum seed cakes were well-dried. The crude fiber content in the cakes ranged from 6.35% to 9.85% (Table 1). Plum seed cakes previously defatted with \(n\)-hexane, chloroform:methanol (2:1 v/v), and acetone had a higher crude fiber content compared to cakes previously defatted with ethyl-acetate (\(p < 0.05\)). Otherwise, plant fiber does not give the body energy, but they are a valuable source of mineral and vitamin components that play an important role in the body. The values of these two parameters (moisture content and crude fiber) obtained in this study are similar to the results obtained
by Čakarević et al. [14]. The moisture content of 10.80% and 6.27% was for the plum seed cakes obtained after cold-pressing and supercritical fluid extraction of oil, respectively. In the same samples, the crude fiber content was 5.86% and 11.57%, respectively. As with plum seed cakes, apricot seed cakes obtained by cold-pressing oil and supercritical fluid extraction of oil had a moisture content of 8.90% and 6.25%, i.e., the crude fiber content of 8.94% and 8.06%, respectively [28].

Table 1. Physico-chemical properties of plum seed cake.

| Property          | n-Hexane       | n-Heptane     | Ethyl-Acetate | Chloroform:Methanol (2:1 v/v) | Acetone           |
|-------------------|----------------|---------------|---------------|-----------------------------|-------------------|
| Moisture (%)      | 7.66 ± 0.23    | 7.09 ± 0.21   | 5.96 ± 0.09   | 7.46 ± 0.11                | 7.47 ± 0.14       |
| Crude fiber (%)   | 9.32 ± 0.19    | 7.95 ± 0.14   | 6.36 ± 0.10   | 9.85 ± 0.18                | 8.39 ± 0.24       |
| Protein (%)       | 57.01 ± 1.70   | 61.90 ± 1.73  | 36.95 ± 1.14  | 46.03 ± 1.01               | 54.55 ± 0.98      |
| pH value          | 5.49 ± 0.11    | 5.33 ± 0.15   | 5.37 ± 0.14   | 5.54 ± 0.15                | 5.53 ± 0.13       |
| Amygdalin (mg/g d.w.) | 0.139 ± 0.002  | 0.041 ± 0.001 | 0.100 ± 0.002 | 0.102 ± 0.003              | 0.005 ± 0.0001    |

Data are expressed as mean ± standard deviation (n = 3). The different letters (lowercase) in the same row indicate the statistically significant between the samples (p < 0.05).

The solvent polarity also had a significant effect on the protein content of the plum seed cake (p < 0.05) (Table 1). The highest protein content was determined in the cake obtained by defatting plum seeds with n-heptane (61.90%), and it was slightly lower for the cake defatted with n-hexane (57.01%) (p < 0.05). The lowest protein content was obtained for plum seed cake defatted with ethyl-acetate (36.95%). Otherwise, this cake was very similar to commercial plum seed flour obtained by cold-pressing of oil, in which the protein content was 36.6%. Čakarević et al. [14] determined a slightly higher protein content of 50.69% for plum seed cake obtained by cold-pressing of oil, while the protein content in the cake defatting by supercritical fluid extraction was 48.93%. The protein content in plum seed cake (38.6%) defatted with n-hexane was lower compared to the literature data [8]. Based on the obtained data, it can be concluded that the extraction procedure and solvent had a significant effect on the protein content in the cake.

The measured pH values of about 5.5 for suspensions (Table 1) indicated that the analyzed plum seed cakes were slightly acidic. The acidic nature of the cake was a consequence of the presence of organic acids that come from the plum seed itself. These values were similar compared to the pH value measured in palm seed cake (pH of about 5.2) [29].

Amygdalin, a cyanogenic glycoside, hydrolyzes into glucose and aglycone in the human body under the action of the enzyme glucosidase. Aglycone is further degraded to benzaldehyde and hydrocyanic acid. The released hydrocyanic acid is rapidly resorbed in the upper gastrointestinal tract and can lead to respiratory arrest. Therefore, amygdalin is considered toxic, and its acute lethal oral dose is 0.5–3.5 mg/kg body weight. In order to assess the possibility of human application of plum seed cake, the content of amygdalin was determined (Table 1). The amygdalin content in the cakes ranged from 0.005 to 0.139 mg/g d.w. The European Food Safety Authority (EFSA) [30] found that an acute reference dose of cyanogenic glycosides in raw apricot kernels was 20 µg/kg body weight. Having in mind these data, the results obtained for the content of amygdalin in plum seed cakes indicate their safe use. Čakarević et al. [14] showed that plum seed cakes obtained from oil extraction by cold pressing and supercritical fluid extraction are safe to use due to their low amygdalin content.

3.2. Functional Properties of Plum Seed Cake

The functional properties of plum seed cakes are shown in Table 2. The interactions of water and oil with plum seed cakes are very important in food systems because of their effect on the taste and texture of food. The WAC value can be affected by various parameters, such as the size and shape of the crushed cake particles, hydrophilic and hydrophobic interactions, and the presence of lipids, carbohydrates, and amino acid residues on the
The WAC of different plum seed cakes was ranged from 1.38 to 1.61 g/g of the sample (Table 2).

**Table 2.** Functional properties of plum seed cake. Data are expressed as mean ± standard deviation (n = 3).

| Properties          | n-Hexane   | n-Heptane | Ethyl-Acetate | Chloroform:Methanol (2:1 v/v) | Acetone   |
|---------------------|------------|-----------|---------------|-------------------------------|-----------|
| WAC (g/g)           | 1.45 ± 0.04<sup>cd</sup> | 1.38 ± 0.03<sup>d</sup> | 1.51 ± 0.03<sup>bc</sup> | 1.61 ± 0.04<sup>a</sup> | 1.59 ± 0.02<sup>ab</sup> |
| WSI (%)             | 3.56 ± 0.10<sup>b</sup> | 3.16 ± 0.09<sup>d</sup> | 3.27 ± 0.08<sup>cd</sup> | 3.87 ± 0.11<sup>a</sup> | 3.45 ± 0.09<sup>bc</sup> |
| OAC (g/g)           | 1.38 ± 0.04<sup>b</sup> | 1.41 ± 0.03<sup>b</sup> | 1.98 ± 0.04<sup>a</sup> | 1.46 ± 0.03<sup>b</sup> | 1.10 ± 0.02<sup>c</sup> |
| FC (%)              | 7.63 ± 0.23<sup>a</sup> | 7.55 ± 0.23<sup>a</sup> | 7.18 ± 0.20<sup>a</sup> | 7.34 ± 0.21<sup>a</sup> | 7.63 ± 0.24<sup>a</sup> |
| FS (%)              | 76.00 ± 2.28<sup>a</sup> | 75.00 ± 2.01<sup>ab</sup> | 72.00 ± 2.08<sup>ab</sup> | 74.00 ± 2.15<sup>ab</sup> | 70.00 ± 2.11<sup>b</sup> |
| EA (%)              | 51.56 ± 1.55<sup>ab</sup> | 48.48 ± 1.45<sup>bc</sup> | 55.12 ± 1.61<sup>a</sup> | 47.06 ± 1.40<sup>c</sup> | 48.57 ± 1.43<sup>bc</sup> |
| ES (%)              | 46.00 ± 1.38<sup>a</sup> | 45.45 ± 1.21<sup>bc</sup> | 50.67 ± 1.42<sup>a</sup> | 44.11 ± 1.19<sup>bc</sup> | 42.38 ± 1.22<sup>c</sup> |

Water Absorption Capacity (WAC), Water Solubility Index (WSI), Oil Absorption Capacity (OAC), Foam Capacity (FC), Foam Stability (FS), Emulsification Activity (EA), Emulsion Stability (ES). Data are expressed as mean ± standard deviation (n = 3). The different letters (lowercase) in the same row indicate the statistically significant between the samples (p < 0.05).

A significant difference in the WAC can be attributed to differences in the chemical composition of the cake (p < 0.05). The content of hydrophilic ingredients (such as carbohydrates) binds more water than proteins and lipids [28]. The chemical composition of the cake determines its low WSI. The lowest value of this parameter (3.16%) was determined for the cake obtained after oil extraction with n-heptane, while the highest value of 3.87% was determined for the cake obtained after oil extraction with chloroform:methanol (2:1 v/v). The difference in the WSI between these two cakes was statistically significant since the p-value was lower than 0.05. To our knowledge, the WAC and WSI of plum seed cake have not yet been described in the literature. The WAC of plum seed cake was lower than palm seed cake (4.06 g/g) [28], oranges (12.25 g/g), and apples (8.54 g/g) [29]. The OAC of the analyzed plum seed cakes ranged from 1.10 to 1.98 g/g (Table 2). The obtained results indicated that the polarity of the solvents affects the OAC. The difference in the OAC is most likely due to the presence of non-polar side chains in the cake, which can bind the side chains of hydrocarbons from oil [31]. In the literature, the OAC of lemon, orange, and grapefruit seed cakes was about 4 g/g [32].

Based on its functional properties, it can be concluded that plum seed cake can be used to improve the volume of bread and stabilize colloidal systems due to the possibility of gel formation [33]. Commercially available plum seed flour, obtained after cold-pressing of oil, is used as a dietary supplement that gives dishes a marzipan flavor. In addition, it can be used in the preparation of cakes, bread, and pastries. It is used like other gluten-free flours, in quantities of a maximum of 40 g per recipe. Gelation is the process of aggregation of denatured protein molecules. However, protein conformations, disulfide bonds, and hydrophobicity play a significant role in gelation [34]. The least gelation concentrations of plum seed cakes are shown in Table 3.

**Table 3.** Least gelation concentration of plum seed cakes in the percentage (w/v).

| Cakes                      | 2%  | 4%  | 6%  | 8%  | 10% |
|----------------------------|-----|-----|-----|-----|-----|
| n-Hexane                   | –   | +   | ++  | +   | +++ |
| n-Heptane                  | –   | +   | ++  | +   | +++ |
| Ethyl-acetate              | –   | +   | ++  | +   | +++ |
| Chloroform:Methanol (2:1 v/v) | –   | +   | ++  | +   | +++ |
| Acetone                    | –   | +   | ++  | +   | +++ |

– No gel; + Weak gel; ++ Strong gel; +++ Very strong gel.
All analyzed plum seed cakes did not form a gel at a concentration of 2% (w/v), while a weak gel was formed at a concentration of 4% (w/v). The cakes formed a strong gel at a concentration of 6% (w/v) and a very strong gel at concentrations of 8% (w/v) and 10% (w/v). With these properties, plum seed cakes with a concentration of over 6% (w/v) can be used in formulations in which gel formation is the goal. The least gelation concentration of plum seed cake determined was similar to the gelation concentration for protein extracted from cherry seeds [35]. However, this concentration was significantly lower compared to the value of the least gelation concentration for cold-pressed lemon, orange, and grapefruit seed cakes (16%) [32]. The least concentration of gelation depends on the type of material, the way it is processed (pressing, application of solvents), as well as the ratio of different ingredients present in plant material (proteins, carbohydrates, and lipids).

The FC of the cake refers to the amount of interstitial surface that it can create, while the FS refers to the ability of the cake to stabilize due to gravitational and mechanical stresses [36]. Foam formation and FS are known to be a function of the type of protein present in the cake, pH, oil seed processing methods, viscosity, and surface tension. All analyzed plum seed cakes had a relatively dense foam with a small volume. The FC values in all cases were higher than 7% (Table 2). The lowest FC was obtained for plum seed cake defatted with ethyl-acetate. The cake defatted with acetone had the lowest FS value of 70%, while the cake defatted with n-hexane had the highest FS of 76%. Having in mind that the p-value was lower than 0.05, the difference in the FC between these two cakes was considered statistically significant. According to the literature data [32], plum seed cakes are not a good foaming agent, but they are relatively stable. The EA and ES of the analyzed samples are shown in Table 2. The lowest EA value of 47.06% was determined for the cake defatted with chloroform:methanol (2:1, v/v), while cakes defatted with n-heptane (48.48%) and acetone (48.57%) had almost the same EA value and a statistically non-significant difference (p > 0.05). The highest EA value (55.12%) was determined in the cake defatted with ethyl-acetate, which also had the highest ES value (50.67%). The lowest ES value (42.38%) was determined for the cake defatted with acetone. The obtained results were similar to the results for lemon (EA 39.8% and ES 44.7%), oranges (EA 45.3% and ES 44%), and grapefruit (EA 44.1% and ES 43.9%) seed cakes [32]. Generally, the emulsifying properties of seed cakes depend on the plant material, the method of seed defatting, and the treatment of the cake itself (enzymatic, temperature, microwave). Enzyme treatment has a significantly greater effect on emulsifying properties compared to thermal treatment (baking) [37].

### 3.3. The Antioxidant Potential of Plum Seed Cake

Interest in antioxidants from defatted cakes is constantly growing, since these compounds in a certain concentration have a beneficial effect on human health [38]. Antioxidants are mainly trapped in hard and insoluble structures (vacuoles, lipoprotein bilayers, lignin, and cell wall) [39], making their extraction from seed cakes difficult by conventional extraction techniques. To overcome the mentioned problem, ultrasound-assisted extraction is used to increase the transfer of mass in plant cell tissues [40]. The TAC in plum seed cakes is shown in Table 4. The highest TAC in the ethanol extract was obtained for the plum seed cake previously defatted with acetone (268.9 mg GAE/100 g d.w.), while the lowest content was determined for the defatted cake by ethyl-acetate (183.6 mg GAE/100 g d.w.) (p < 0.05). The TAC in the non-defatted plum seed extract prepared by ultrasound-assisted extraction with 50% (v/v) ethanol at 50 °C, at a liquid-to-solid ratio of 10 mL/g, was 198.03 mg GAE/100 g d.w. [6]. This value was similar to the value obtained for the defatted cake with ethyl-acetate.
Table 4. The content of bioactive compounds identified in the plum seed cakes.

| Compound     | λ (nm) | tR (min) | n-Hexane     | n-Heptane     | Ethyl-Acetate | Chloroform/Methanol (2:1 v/v) | Acetone |
|--------------|--------|----------|--------------|--------------|---------------|--------------------------------|---------|
| Rutin        | 254    | 57.48    | 4.87 ± 0.12 \(\text{a}^a\) | 4.68 ± 0.09 \(\text{a}^a\) | 3.340 ± 0.09 \(\text{c}^c\) | 3.97 ± 0.06 \(\text{b}^b\) | 4.12 ± 0.05 \(\text{b}^b\) |
| Gallic acid  | 278    | 5.38     | 0.53 ± 0.01 \(\text{a}^a\) | 0.49 ± 0.01 \(\text{a}^a\) | 0.360 ± 0.01 \(\text{b}^b\) | 0.42 ± 0.05 \(\text{b}^b\) | 0.50 ± 0.02 \(\text{c}^c\) |
| Syringic acid| 278    | 37.58    | 0.93 ± 0.01 \(\text{b}^b\) | 1.31 ± 0.02 \(\text{a}^a\) | 0.500 ± 0.01 \(\text{d}^d\) | 0.45 ± 0.06 \(\text{d}^d\) | 0.67 ± 0.03 \(\text{c}^c\) |
| Epicatechin  | 278    | 40.31    | 1.12 ± 0.03 \(\text{a}^a\) | 1.08 ± 0.02 \(\text{ab}^b\) | 0.890 ± 0.02 \(\text{c}^c\) | 0.96 ± 0.10 \(\text{bc}^b\) | 1.03 ± 0.04 \(\text{ab}^b\) |
| Caffeic acid | 300    | 30.92    | 0.39 ± 0.01 \(\text{c}^c\) | 0.44 ± 0.01 \(\text{b}^b\) | 0.630 ± 0.01 \(\text{a}^a\) | 0.31 ± 0.01 \(\text{d}^d\) | 0.29 ± 0.01 \(\text{e}^e\) |
| Coumaric acid| 300    | 46.12    | n.d.         | n.d.         | 11.32 ± 0.04 \(\text{c}^c\) | 12.23 ± 0.38 \(\text{b}^b\) | 12.98 ± 0.19 \(\text{a}^a\) |
| TAC          | 765    | -        | 249.90 ± 7.50 \(\text{bc}^b\) | 256.50 ± 7.20 \(\text{ab}^b\) | 183.60 ± 4.60 \(\text{d}^d\) | 234.10 ± 6.30 \(\text{c}^c\) | 268.90 ± 7.80 \(\text{a}^a\) |

n.d.—not detected. Data are expressed as mean ± standard deviation (\(n = 3\)). The different letters (lowercase) in the same row indicate the statistically significant between the samples (\(p < 0.05\)).

Using the RP-HPLC method, the identified and quantified phenolic compounds in ethanolic extracts are depicted in Table 4. Phenolic acids were identified, among which coumaric acid was the most common compound (11.31–12.98 mg/100 g d.w.). The presence of this acid was not only confirmed in the samples previously defatted with n-hexane and n-heptane. Gallic acid (0.36–0.53 mg/100 g d.w.), syringic acid (0.45–1.31 mg/100 g d.w.) and caffeic acid (0.29–0.63 mg/100 g d.w.) were identified in smaller amount. The content of rutin was in the range of 3.34–4.87 mg/100 g d.w. Its highest content was confirmed in the samples previously defatted with n-hexane and n-heptane. Epicatechin had a content between 0.89 and 1.12 mg/100 g d.w. The defatted plum seed cakes are a rich source of phenolic acids, rutin, and epicatechin, which are known to have a positive effect on human health. Khallouki et al. [7] also confirmed the presence of gallic acid (0.1 mg/kg) and syringic acid (0.63 mg/kg) in the methanolic extract of plum seeds previously defatted with n-hexane.

3.4. Antioxidant Activity of Plum Seed Cakes

In order to evaluate the antioxidant potential of ethanolic extracts of defatted plum seed cakes, an in vitro test was applied: testing the ability to neutralize DPPH* The \(\text{IC}_{50}\) values of the analyzed extracts were obtained by interpolation and are depicted in Table 5.

Table 5. Antioxidant activity of various plum seed cake extracts.

| Cakes                   | \(\text{IC}_{50}\) (mg/mL) | Concentration (mg/mL) |
|-------------------------|--------------------------|-----------------------|
| n-Hexane                | 0.48 ± 0.01 \(\text{c}^c\) | 0.0079–2.0240         |
| n-Heptane               | 0.61 ± 0.02 \(\text{b}^b\) | 0.0086–2.1977         |
| Ethyl-acetate           | 0.40 ± 0.01 \(\text{d}^d\) | 0.0024–0.6017         |
| Chloroform:Methanol (2:1 v/v) | 0.65 ± 0.02 \(\text{a}^a\) | 0.0064–1.6342         |
| Acetone                 | 0.63 ± 0.01 \(\text{ab}^b\) | 0.0076–1.9366         |

Data are expressed as mean ± standard deviation (\(n = 3\)). The different letters (lowercase) in the same row indicate statistical significance between the samples (\(p < 0.05\)).

The antioxidant activity of ethanolic extracts of plum seed cake decreased in the following order of applied solvents: ethyl acetate > n-hexane > n-heptane > acetone > chloroform: methanol (2:1 v/v). This order of decreasing the antioxidant activity of cake extracts is also expected, having in mind the activity of oils obtained from plum seeds using these solvents [6]. Oils extracted by polar solvents showed better antioxidant activity. On that occasion, those compounds were extracted from plum seeds that are responsible for this activity. In that case, compounds with antioxidant activity remained in the cake with a lower content, which was shown during this research. Khallouki et al. [7] determined an \(\text{IC}_{50}\) value of 0.33 mg/mL for the methanolic extract of defatted plum seed. Compared with this result, ethanolic extracts of plum seed cake previously defatted with ethyl-acetate had similar activity. The activity of other extracts was lower (\(p < 0.05\)). However, plum seed cake extracts can be considered a valuable natural source of antioxidants. As such, they can...
be used in the diet as a functional food, which potentially reduces the symptoms of chronic diseases, in the first place those that are caused by oxidative stress (neurodegenerative and cardiovascular diseases).

4. Conclusions

After oil isolation from plum seeds, a significant amount of cakes are remained in the food industry. Because of that, the physico-chemical and functional properties of cakes were investigated in this study. The low WAC and moderate OAC of the cake were important factors for products in which hydration and viscosity improvement are necessary. Due to the possibility of gelation, the cakes can be used in formulations in which the creation of a gel is the final goal. The cakes can be considered safe to use because the amygdalin content was in the allowed range. The presence of antioxidants was also confirmed in the analyzed samples. Having in mind that plum seed cakes were enriched in protein, crude fiber and antioxidants, the cakes could be used as a raw material for the development of sustainable functional products. Future research should be focused on the explanation of the mechanisms of cake extracts action should be determined, which would contribute to an even better understanding of pharmacological activities.

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References

1. El Barnossi, A.; Moussaid, F.; Housseini, A.I. Tangerine, banana and pomegranate peels valorisation for sustainable environment: A Review. Biotechnol. Rep. 2021, 29, e00574. [CrossRef] [PubMed]

2. Available online: https://www.atlasbig.com/en-gb/countries-by-plum-production (accessed on 26 June 2022).

3. Dołżyńska, M.; Obidziński, S.; Piekut, J.; Yıldız, G. The utilization of plum stones for pellet production and investigation of post-combustion flue gas emissions. Energies 2020, 13, 5107. [CrossRef]

4. Kamel, B.S.; Kakuda, Y. Characterization of the seed oil and meal from apricot, cherry, nectarine, peach and plum. J. Am. Oil Chem. Soc. 1992, 69, 492–494. [CrossRef]

5. Djuricic, I.; Calder, P.C. Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: An update for 2021. Nutrients 2021, 13, 2421. [CrossRef] [PubMed]

6. Savic, I.; Savic Gajic, I.; Gajic, D. Physico-chemical properties and oxidative stability of fixed oil from plum seeds (Prunus Domestica Linn.). Biomolecules 2020, 10, 294. [CrossRef]

7. Khallouki, F.; Haubner, R.; Erben, G.; Ulrich, C.M.; Owen, R.W. Phytochemical compositon and antioxidant capacity of various botanical parts of the fruits of Prunus Domestica L. from the Lorraine region of Europe. Food Chem. 2012, 133, 697–706. [CrossRef]

8. Gonzalez-Garcia, E.; Marina, M.L.; Garcia, M.C. Plum (Prunus domestica L.) by-product as a new and cheap source of bioactive peptides: Extraction method and peptides characterization. J. Funct. Foods 2014, 11, 428–437. [CrossRef]

9. Milala, J.; Kosmala, M.; Sójka, M.; Kołodziejczyk, K.; Zbuzenziak, M.; Markowski, J. Plum pomaces as a potential source of dietary fibre: Composition and antioxidant properties. J. Food Sci. Technol. Mys. 2013, 50, 1012–1017. [CrossRef]

10. Savić, I.M.; Nikolić, V.D.; Savić-Gajić, I.M.; Kundaković, T.D.; Stanojković, T.P.; Najman, S.J. Chemical composition and biological activity of the plum seed extract. Ato. Technol. 2016, 5, 38–45. [CrossRef]

11. Sójka, M.; Kołodziejczyk, K.; Milala, J.; Abadias, M.; Viñas, I.; Guyot, S.; Baron, A. Composition and properties of the polyphenolic extracts obtained from industrial plum pomines. J. Funct. Foods 2015, 12, 168–178. [CrossRef]

12. Mirpoor, S.F.; Giosafatto, C.V.L.; Porta, R. Biorefining of seed oil cakes as industrial co-streams for production of innovative bioplastics. A Review. Trends Food Sci. Technol. 2021, 109, 259–270. [CrossRef]
13. Kaur, M.; Singh, B.; Kaur, A.; Singh, N. Proximate, mineral, amino acid composition, phenolic profile, antioxidant and functional properties of oilseed cakes. *Int. J. Food Sci. Technol.* 2021, 56, 6732–6741. [CrossRef]

14. Čakarević, J.C.; Vidović, S.S.; Vladić, J.Z.; Jokić, S.D.; Pavlović, N.S.; Popović, L.M. Plum oil cake protein isolate: A potential source of bioactive peptides. *Food Feed Res.* 2019, 46, 171–178. [CrossRef]

15. AOAC. *Official Methods of Analysis, Association of Official Agricultural Chemists;* AOAC: Washington, DC, USA, 2000.

16. Bolariinwa, I.F.; Orfila, C.; Morgan, M.R. Amygdalin content of seeds, kernels and food products commercially-available in the *UK. Food Chem.* 2014, 152, 133–139. [CrossRef]

17. Lin, M.J.Y.; Humbert, E.S.; Sosulski, F.W. Certain functional properties of sunflower meal products. *J. Food Sci.* 1974, 39, 368–370. [CrossRef]

18. Kompaoré, W.R.; Nikièma, P.A.; Bassolé, H.I.N.; Savadogo, A.; Moueucoucou, J. Chemical composition and antioxidative properties of seeds of *Moringa Oleifera* and pulps of *Parkia Biglobosa* and *Adansonia Digitata* commonly used in food fortification in burkina faso. *Curr. Res. J. Biol. Sci.* 2011, 3, 64–72.

19. Onwulata, C.I.; Konstance, R.P.; Smith, P.W.; Holsinger, V.H. Physical properties of extruded products as affected by cheese whey. *Int. J. Food Sci. Technol.* 1977, 12, 473–484. [CrossRef]

20. Coffmann, C.W.; Garciaj, V.V. Functional properties and amino acid content of a protein isolate from mung bean flour. *Int. J. Food Sci. Technol.* 1998, 33, 814–818. [CrossRef]

21. Lawhon, J.T.; Cater, C.M.; Mattil, K.F. Comparative study of the whipping potential of an extract from several oilseed flours. *J. Cereal Sci.* 1972, 17, 240–244. [CrossRef]

22. Savic, I.M.; Savic Gajic, I.M. Optimization study on extraction of antioxidants from plum seeds (*Prunus domestica* L.). *Optim. Eng.* 2021, 22, 141–158. [CrossRef]

23. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent. In *Methods in Enzymology;* Academic Press: Cambridge, MA, USA, 1999; Volume 299, pp. 152–178.

24. Nour, V.; Trandafir, I.; Cosmulescu, S. HPLC determination of phenolic acids, flavonoids and juglone in walnut leaves. *J. Chromatogr. Sci.* 2013, 51, 883–890. [CrossRef] [PubMed]

25. Savic Gajic, I.; Savic, I.; Boskovic, I.; Zerajic, S.; Markovic, I.; Gajic, D. Optimization of ultrasound-assisted extraction of phenolic compounds from black locust (*Robinia pseudoacacia*) flowers and comparison with conventional methods. *Antioxidants* 2019, 8, 248. [CrossRef] [PubMed]

26. Tan, Z.J.; Yang, Z.Z.; Yi, Y.J.; Wang, H.Y.; Zhou, W.L.; Li, F.F.; Wang, C.Y. Extraction of oil from flaxseed (*Linum usitatissimum* L.) using enzyme-assisted three-phase partitioning. *Appl. Biochem. Biotechnol.* 2016, 179, 1325–1335. [CrossRef] [PubMed]

27. Nas, S.; Gökalp, H.Y.; Unsal, M. *Vegetable Oil Technology;* Faculty of Engineering, Pamukkale University, Textbooks Publication: Pamukkale, Turkey, 2001.

28. Čakarević, J.; Vidović, S.; Vladić, J.; Gavarić, A.; Jokić, S.; Pavlović, N.; Blažić, M.; Popović, L. Production of bio-functional protein through revalorization of apricot kernel cake. *Foods* 2019, 8, 318. [CrossRef] [PubMed]

29. Majzoobi, M.; Karambaksh, G.; Golmankani, M.T.; Mesbahi, G.R.; Farahnaki, A. Chemical composition and functional properties of date press cake, an agro-industrial waste. *J. Agr. Sci. Technol.-Iran* 2019, 21, 1807–1817.

30. (EFSA) EFSA Panel on Contaminants in the Food Chain. Acute health risks related to the presence of cyanogenic glycosides in raw apricot kernels and products derived from raw apricot kernels. *EFSA J.* 2016, 14, 4424.

31. Olti, N.; Ktenioudaki, A.; Smyth, T.P.; McLoughlin, P.; Doran, L.; Auty, M.A.E.; Gallagher, E. Physicochemical Assessment of two fruit by-products as functional ingredients: Apple and orange pomace. *J. Food Eng.* 2015, 153, 89–95. [CrossRef]

32. Adebowale, K.O.; Lawal, O.S. Comparative study of the functional properties of bambara groundnut (*Voandzeia Subterrannea*), jack bean (*Canavalia Ensiformis*) and mucuna bean (*Mucuna Pruriens*) flours. *Food Res. Int.* 2004, 37, 355–365. [CrossRef]

33. Karaman, E.; Karabiber, E.B.; Yılmaz, E. Physicochemical and functional properties of the cold press lemon, orange, and grapefruit seed meals. *Qual. Assur. Saf. Crop.* 2018, 10, 233–243. [CrossRef]

34. Olaofe, O.; Adeyemi, F.O.; Adeediran, G.O. Amino acid and mineral compositions and functional properties of some oilseeds. *J. Agr. Food Chem.* 1994, 42, 878–881. [CrossRef]

35. Olafode, O.; Adeyemi, F.O.; Adeediran, G.O. Amino acid and mineral compositions and functional properties of some oilseeds. *J. Food Sci. Technol. Mys.* 2018, 63, 814–818. [CrossRef]

36. Olaofe, O.; Adeyemi, F.O.; Adediran, G.O. Amino acid and mineral compositions and functional properties of some oilseeds. *J. Food Sci. Technol. Mys.* 2018, 63, 814–818. [CrossRef]

37. Teh, S.S.; Birch, E.J. Effect of ultrasonic treatment on the polyphenol content and antioxidant capacity of extract from defatted hemp, flax and canola seed cakes. *Ultrasound. Sonochim.* 2014, 21, 346–353. [CrossRef]