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Original Research

Freeze-Dried Encapsulation of Phenolic Compounds of Chokeberry Juice in Whey Proteins in the Presence of Lecithin

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Abstract

Background: Microencapsulation is an emerging technique to ensure the stability and viability of polyphenols and requires a carrier that may also provide health benefits. With this in mind, chokeberry juice, a source of polyphenols known for its antioxidant potential and antimicrobial and anti-inflammatory actions, was used along with whey proteins. **Methods:** Complexes were prepared by complexing chokeberry juice with carriers: whey protein or whey protein in combination with lecithin. After complexation, the samples were freeze-dried to obtain dry powders. The samples were analyzed for total polyphenol and proanthocyanidin contents, as well as concentrations of individual polyphenols. To evaluate the antioxidant potential of the complexes, DPPH, ABTS, FRAP, and CUPRAC methods were used. Additionally, color parameters and IR spectra of the carriers and corresponding complexes were determined. **Results:** The results showed that lecithin affected polyphenol adsorption onto the carriers. Overall, whey proteins showed higher affinity for adsorbing chokeberry juice polyphenols, except for quercetin. IR spectra screening confirmed that chokeberry polyphenols adsorbed onto the carriers and altered their structure. **Conclusions:** This method of complex preparation offers a green approach to developing functional food additives that enrich products with both polyphenols and proteins while improving product stability by increasing antioxidant potential and potentially modifying color.

Keywords: chokeberry juice; polyphenols; whey protein; whey protein in combination with lecithin; freeze-drying

1. Introduction

Berry intake has received considerable attention in global dietary guidelines because of the associated high phytochemical content, which plays a significant role in mitigating the impact of noncommunicable disorders [1,2,3,4]. Among these fruits, chokeberries (*Aronia melanocarpa*) are recognized as a particularly rich source of bioactive compounds, specifically anthocyanins, flavonoids, and proanthocyanidins [1,2,3]. In chokeberry juice, in addition to cyanidin-3-galactoside, cyanidin-3-arabinoside is a major anthocyanin pigment, and among quercetin derivatives, quercetin-3-rutinoside is the most important. Among chokeberry juice phenolic acids, chlorogenic and neochlorogenic acids are considered the most important [5]. Over time, numerous investigations have linked phenolic compounds to potential health benefits, most notably antioxidant potential, antimicrobial properties, anti-inflammatory activity, anticancer potential, regulation of cardiovascular disorders, balancing of lipid profiles, and possible regulation of diabetes and hyperglycemia [6,7,8,9,10,11,12]. Despite these health benefits, their instability is a major drawback. Over the years, researchers have employed many techniques to overcome their sensitivity to environmental factors, thereby enhancing stability and overall bioavailability [13]. Nowadays, developing synergistic food systems that strategically combine chokeberry polyphenols with complementary structure-forming ingredients offers a proactive approach to enhance structural stability, facilitate targeted bioaccessibility, and support the formulation of bioactive food additives [14]. Microencapsulation is an emerging processing technique to ensure polyphenol stability and viability, requiring an adequate carrier [15] and, more recently, carriers with potential health benefits. Consequently, creating these bioactive food additives serves as a primary baseline for further exploration. Among various encapsulation techniques, freeze-drying was selected because its low temperatures help preserve polyphenol stability, especially anthocyanins [15], minimizing loss during the production of bioactive food additives. Whey proteins were chosen as carriers because of their health-promoting attributes. Indeed, whey protein molecules are amphiphilic, which can help formulate edible carrier systems to improve the performance of bioactive compounds [16]. In their review, the authors emphasize the potential of these carrier systems as encapsulating agents that protect and deliver bioactive compounds in the food, cosmetic, and pharmaceutical industries [16]. Whey proteins, a byproduct of cheese production, comprise several fractions, including β-lactoglobulin, α-lactoglobulin, bovine serum albumin, and various immunoglobulins. The potential to use whey proteins in various carrier formulations largely stems from their surface

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characteristics, such as electrical charge, hydrophobicity, and chemical reactivity [17]. These characteristics can be altered by modifying solution or environmental conditions, such as ionic strength, pH, and temperature, allowing control over molecular interactions between whey proteins. These changes can lead to attractive or repulsive protein interactions, causing them to form diverse structures such as sheets, spheres, or fibers [16,18].

These changes can also affect interactions between whey proteins and bioactive compounds, specifically chokeberry juice polyphenols in this research. The presence of other compounds during carrier construction, or the complexation or conjugation of whey proteins with compounds such as polysaccharides, lipids, and other proteins, can enhance or diminish whey protein functionality. In our study, this additional compound was lecithin. Currently, there is strong demand for effective solutions for encapsulating bioactive compounds, and lecithin-based systems are increasingly desirable because of the biocompatibility and amphiphilic self-assembly behavior of lecithin [19]. Similar to whey protein, lecithin is recognized for its health benefits and its uses in the food industry as a stabilizer, wetting agent, emulsifier, and antioxidant [20].

To our knowledge, no study has investigated encapsulating chokeberry juice polyphenols using whey proteins alone or in combination with lecithin, nor has any study compared these two carriers or examined how lecithin affects polyphenol adsorption. Thus, this study evaluated the impact of lecithin on the adsorption of chokeberry juice polyphenols onto whey proteins using a freeze-dried encapsulation process, compared with pure whey proteins. The resulting freeze-dried powders were assessed for total polyphenol content, proanthocyanidin content, and antioxidant activity using spectrophotometric assays, and high-performance liquid chromatography (HPLC) was used to determine individual polyphenol concentrations. Additionally, color parameters and infrared (IR) spectra were recorded.

2. Materials and Methods

2.1 Preparation of Freeze-Dried Complexes

Complexation of chokeberry juice with a protein matrix was performed to prepare complexes. A total of 2 g of the protein matrix (whey proteins or whey proteins in combination with lecithin, both obtained from Glanbia Nutritionals, Orsingen-Nenzingen, Germany) was added to 40 mL of chokeberry juice. The mixtures were agitated at room temperature on a magnetic stirrer for 15 minutes. The resulting mixtures were then centrifuged at 8000 rpm (8586 ×g) for 15 minutes. The precipitate was freeze-dried, while the separated supernatant was used to evaluate adsorption capacity, as described in Section 2.4. The precipitates were first frozen at −18 °C for 24 hours and then freeze-dried using a laboratory freeze-dryer (Christ Freeze Dryer, Alpha 1–4, Osterode am Harz, Germany). The selected freeze-drying parameters were a freezing temperature of −55 °C, a sublimation temperature from −35 °C to 0 °C under a vacuum of 0.220 mbar, and an isothermal desorption temperature from 0 °C to 21 °C under a vacuum of 0.060 mbar. After 6 hours of freeze-drying, two types of dried powders, i.e., complexes, were obtained: WL_C: complexes based on whey proteins in combination with lecithin, enriched with chokeberry juice polyphenols; W_C: complexes based on whey proteins, enriched with chokeberry juice polyphenols. Two batches of each complex were prepared.

2.2 Ultrasound-Assisted Extraction of Freeze-Dried Complexes

Ultrasound-assisted extraction of complexes was performed. First, 0.5 g of complexes were weighed into a plastic tube and mixed with 10 mL of acidified methanol (methanol acidified with concentrated HCl at a methanol:acid ratio of 99:1) as the extraction solvent. The prepared samples were placed in an ultrasonic bath (Bandelin, Berlin, Germany) for 20 minutes at 25 °C, with a nominal ultrasonic power of 80 W and a frequency of 35 kHz. After ultrasonic treatment, the samples were centrifuged at 8000 rpm (8586 ×g) for 15 minutes. The resulting supernatants (i.e., extracts of protein complexes) were collected and used for further analysis of polyphenols and antioxidant activity by spectrophotometric and HPLC methods.

2.3 Estimation of Total Polyphenols, Proanthocyanidins and Monomeric Anthocyanins

All parameters were determined spectrophotometrically using a UV-V is spectrophotometer (Cary 60, Agilent Technologies, Santa Clara, CA, USA) to measure absorbance. The Folin–Ciocalteu method was used to estimate total polyphenols, as described by Singleton and Rossi [21]. The DMAC method was used to evaluate proanthocyanidins, as described by Prior et al. [22]. Results were calculated from calibration curves and expressed as mg of gallic acid equivalents per g of complexes (mg GAE/g) and mg of procyanidin B2 equivalents per g of complexes (mg PB2E/g), respectively. The pH differential method, as specified by Giusti and Wrolstad [23], was used to estimate monomeric anthocyanins. All experiments were repeated three times.

2.4. Calculation of Adsorption Capacity

To estimate the affinity of chokeberry juice polyphenols for protein matrices, the adsorption capacity was calculated using the following equation, based on total polyphenol and monomeric anthocyanin results:

$$\mathrm{AC}\ (\%) = ((\mathrm{ccm} - \mathrm{cs}) / \mathrm{ccm}) \times 100$$

where AC is the adsorption capacity, ccm is the concentration of compounds (i.e., total polyphenols or monomeric anthocyanins) in the complexation mixture, and cs is the concentration of compounds (i.e., total polyphenols or monomeric anthocyanins) in the supernatant.

2.5 Estimation of Individual Polyphenols by HPLC Analysis

HPLC analysis was performed to qualitatively and quantitatively determine individual polyphenols. A 1260 Infinity II HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a vial sampler, quaternary pump, diode array detector (DAD), and a Poroshell 120 EC C-18 column (4.6 × 100 mm, 2.7 µm) was used. Before injection, extracts obtained by ultrasound-assisted extraction were filtered through polytetrafluoroethylene (PTFE) filters (0.20 µm pores). Then, 10 µL of the filtered extracts were injected at a flow rate of 1.0 mL/min. The column temperature was set to 28 °C. The DAD monitored UV-vis spectra from 190 to 600 nm. The mobile phases for gradient elution of polyphenols were 0.1% aqueous orthophosphoric acid (mobile phase A) and 100% methanol (mobile phase B). The elution gradient used to separate individual polyphenols was as follows: 0 min, 5% B; 3 min, 30% B; 15 min, 35% B; 22 min, 37% B; 30 min, 41% B; 32 min, 45% B; 40 min, 49% B; 45 min, 80% B; 48 min, 80% B; 50 min, 5% B; 53 min, 5% B. UV-Vis spectra were recorded on the DAD detector in the interval from 190 nm to 600 nm.

Polyphenols were identified by retention time and UV-vis spectra compared with standards. Anthocyanins were measured at 520 nm, phenolic acids at 280 nm, and quercetin at 360 nm. Calibration curves were prepared for each compound to quantify individual polyphenols, and results were expressed as mg of compound per 100 g of sample (mg/100 g), except for the chlorogenic acid derivative, which was expressed as chlorogenic acid. Calibration curves were prepared for cyanidin 3-galactoside (1–500 mg/L; $r^2 = 0.9998$; LOD = 0.0006 mg/L; LOQ = 0.0019 mg/L); cyanidin 3-arabinoside (1–150 mg/L; $r^2 = 0.9996$; LOD = 0.29 mg/L; LOQ = 0.89 mg/L); quercetin (1–150 mg/L; $r^2 = 0.9998$; LOD = 0.605 mg/L; LOQ = 1.83 mg/L); neochlorogenic acid (5–1500 mg/L; $r^2 = 0.9996$; LOD = 0.71 mg/L; LOQ = 2.15 mg/L); chlorogenic acid (5–1000 mg/L; $r^2 = 0.9997$; LOD = 0.0002 mg/L; LOQ = 0.0006 mg/L).

2.6 Estimation of Antioxidant Activity

Antioxidant activity was estimated using the cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), free radical scavenging capacity (DPPH), and total antioxidant capacity (ABTS) methods, with absorbance measured on a UV-vis spectrophotometer (Cary 60, Agilent Technologies, Santa Clara, CA, USA). Results were calculated from the calibration curve and expressed as µmol of Trolox equivalents per 100 g of complexes (µmol TE/100 g). All experiments were repeated three times. Apak et al. [24] described the CUPRAC method in detail. Benzie and Strain [25] described the FRAP method. Brand-Williams et al. [26] described the DPPH method, and Arnao et al. [27] described the ABTS method.

2.7 Recording of IR Spectra

The IR instrument (Cary 630) with MicroLab Expert software (both from Agilent Technologies, Santa Clara, CA, USA) was used to screen protein matrices and the corresponding complexes by FTIR-ATR (Fourier transform infrared spectroscopy–attenuated total reflectance). The instrument contains an ATR crystal onto which the sample was placed and pressed. IR spectra were recorded from 4000 to 600 cm^{-1} at a resolution of 4 cm^{-1}, with 32 scans collected. Second-derivative spectra were also obtained. Screening was conducted at room temperature.

2.8 Color Measurement

Color parameters were measured using a chromameter (Minolta CR-400, Konica Minolta, Inc., Osaka, Japan). Results were expressed in the CIELAB color space, with L* (lightness), a* (redness (+) and greenness (−)), and b* (yellowness (+) and blueness (−)). The °h and C* parameters were also recorded. All measurements were performed in triplicate.

2.9 Statistical Analysis

Results are presented as the mean ± standard deviation (SD). Statistical analysis was performed using Statistica 13.1 (StatSoft, Tulsa, OK, USA) with one-way analysis of variance (ANOVA) and Fisher's least significant difference test at a significance level of $p < 0.05$.

3. Results

3.1 Freeze-Dried Complexes Polyphenols Profile and Antioxidant Activity

Adsorption capacity was calculated to estimate and compare the adsorption affinity of total polyphenols and monomeric anthocyanins for whey proteins and for whey proteins in combination with lecithin (Fig. 1). Whey proteins with lecithin showed slightly higher adsorption affinity for total polyphenols than whey proteins alone (75.6% vs. 72.2%). However, this trend was reversed for monomeric anthocyanins, for which whey proteins with lecithin had lower adsorption affinity than whey proteins alone (31.8% vs. 54.8%).

Characteristics of chokeberry juice that was used for complexation with protein matrices are presented in Table 1. The results of estimation of total polyphenols content and proanthocyanidins content, as well as antioxidant activity of the formulated complexes are presented in Table 2. From the results, it can be observed that whey complexes contained higher contents of both total polyphenols

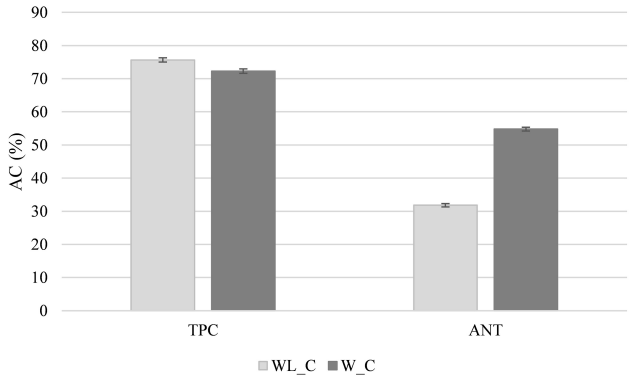


Fig. 1. Adsorption capacity (AC) of protein matrices for total polyphenols and monomeric anthocyanins. (WL_C – complexes based on whey proteins in combination with lecithin enriched with chokeberry juice polyphenols; W_C – complexes based on whey proteins enriched with chokeberry juice polyphenols).

and proanthocyanidins than whey with lecithin complexes. Whey complexes had 59.46 mg GAE/g of total polyphenols and 54.79 mg PB2E/g of proanthocyanidins, compared to 51.41 mg GAE/g of total polyphenols and 42.85 mg PB2E/g of proanthocyanidins for whey with lecithin complexes. With all selected methods for measuring antioxidant activity, the same pattern was noted as for total polyphenols and proanthocyanidins, i.e., whey complexes exhibited higher antioxidant activity than whey with lecithin complexes. Specifically, the antioxidant activities of whey complexes were 255.92 µmol TE/100 g, 354.91 µmol TE/100 g, 4.21 µmol TE/100 g, and 3.11 mmol TE/100 g for the DPPH, ABTS, FRAP, and CUPRAC methods, respectively. For whey with lecithin complex, these values were 204.66 µmol TE/100 g, 315.22 µmol TE/100 g, 3.34 µmol TE/100 g, and 2.35 mmol TE/100 g for the DPPH, ABTS, FRAP, and CUPRAC methods, respectively.

Concentrations of individual polyphenols determined by HPLC are presented in Table 3, and a typical HPLC chromatogram for the investigated complexes is shown in Fig. 2. Not all polyphenols followed this trend. Two anthocyanins were identified in the complexes, cyanidin 3-galactoside and cyanidin 3-arabinoside. Both were found at higher concentrations in whey complexes (273.76 mg/100 g and 75.53 mg/100 g) than in whey with lecithin complexes (234.76 mg/100 g and 63.74 mg/100 g). Quercetin was found at a significantly higher concentration in whey with lecithin complexes (118.09 mg/100 g) than in whey complexes (91.07 mg/100 g). Chlorogenic and neochlorogenic acids followed the trend observed for anthocyanins and were measured at significantly higher concentrations in whey complexes. Whey complexes contained 318.54 mg/100 g and 174.94 mg/100 g, while whey with lecithin complexes contained 235.07 mg/100 g and 122.33 mg/100 g of these phenolic acids. For the third phenolic acid, a derivative of chlorogenic acid, the difference between complexes was less pronounced than for the previous phenolic acids. Whey complexes contained 125.75 mg/100 g, while whey with lecithin complexes contained 121.02 mg/100 g of this derivative.

Table 1. Characteristics of chokeberry juice used for complexation.

TPC (g GAE/L)	25.35 ± 0.17
PAC (g PB2E/L)	8.56 ± 0.07
DPPH (µmol TE/100 mL)	240.45 ± 0.39
ABTS (µmol TE/100 mL)	345.85 ± 0.45
FRAP (µmol TE/100 mL)	15.89 ± 0.43
CUPRAC (mmol TE/100 mL)	3.056 ± 0.45
Cyanidin 3-galactoside (mg/L)	612.80 ± 0.47
Cyanidin 3-arabinoside (mg/L)	117.94 ± 0.21
Quercetin (mg/L)	182.15 ± 0.23
Neochlorogenic acid (mg/L)	974.65 ± 1.28
Chlorogenic acid (mg/L)	742.15 ± 1.14
pH	3.72 ± 0.01
Total soluble solids (%)	15.2 ± 0.01

TPC, total polyphenol content; GAE, gallic acid equivalents; PAC, proanthocyanidins; PB2E, procyanidin B2 equivalents; DPPH, free radical scavenging capacity; ABTS, total antioxidant capacity; FRAP, ferric reducing antioxidant power; CUPRAC, cupric reducing antioxidant capacity.

Table 2. Contents of total polyphenols (TPC) and proanthocyanidins (PAC) and antioxidant activity of complexes.

	WL_C	W_C
TPC (mg GAE/g)	51.41 ± 0.68^{b}	59.46 ± 0.61^{a}
PAC (mg PB2E/g)	42.85 ± 0.16^{b}	54.79 ± 0.12^{a}
DPPH (µmol TE/100 g)	204.66 ± 0.57^{b}	255.92 ± 0.76^{a}
ABTS (µmol TE/100 g)	315.22 ± 0.38^{b}	354.91 ± 0.86^{a}
FRAP (µmol TE/100 g)	3.34 ± 0.02^{b}	4.21 ± 0.03^{a}
CUPRAC (mmol TE/100 g)	2.35 ± 0.02^{b}	3.11 ± 0.02^{a}

WL_C – complexes based on whey proteins in combination with lecithin enriched with chokeberry juice polyphenols; W_C – complexes based on whey proteins enriched with chokeberry juice polyphenols. Different letters (a and b) represent values that are statistically different.

3.2 Color Parameters of Protein Matrices and Freeze-Dried Complexes

Table 4 compares the color parameters of carriers and formulated complexes. In contrast, photographs of carriers and resulting complexes enriched with chokeberry juice polyphenols are shown in Fig. 3. Whey protein had a higher L* value than whey protein with lecithin (94.01 vs. 90.50). Upon adsorption of chokeberry polyphenols, the L* values, which represent lightness, decreased signif-

Table 3. Individual polyphenols determined by HPLC (mg/100 g) in complexes.

	WL_C	W_C
Cyanidin 3-galactoside	234.76 ± 2.68^{b}	273.76 ± 0.71^{a}
Cyanidin 3-arabinoside	63.74 ± 0.71^{b}	75.53 ± 1.11^{a}
Quercetin	118.09 ± 3.96^{a}	91.07 ± 3.23^{b}
Neochlorogenic acid	122.33 ± 0.58^{b}	174.94 ± 1.26^{a}
Derivative of chlorogenic acid	121.02 ± 0.27^{b}	125.75 ± 0.13^{a}
Chlorogenic acid	235.07 ± 3.35^{b}	318.54 ± 0.71^{a}

WL_C – complexes based on whey proteins in combination with lecithin enriched with chokeberry juice polyphenols; W_C – complexes based on whey proteins enriched with chokeberry juice polyphenols. Different letters (a and b) represent values that are statistically different.

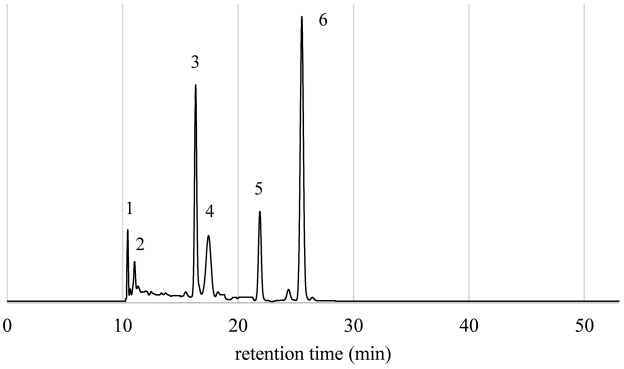


Fig. 2. Typical HPLC chromatogram for extracts of investigated complexes. (1 - cyanidin 3-galactoside, 2 - cyanidin 3-arabinoside, 3 - chlorogenic acid, 4 - derivative of chlorogenic acid, 5 - neochlorogenic acid, 6 - quercetin). HPLC, high-performance liquid chromatography.

icantly, indicating that the complexes became darker. The L* values were 52.66 and 42.60 for whey and whey with lecithin complexes, respectively. The a* value, which represents the redness of samples, was 0.10 for whey and –1.57 for whey protein with lecithin. Adsorption of chokeberry polyphenols, particularly anthocyanins, caused a significant increase in redness; thus, whey and whey with lecithin complexes had a* values of 17.87 and 12.34, respectively. A similar pattern was observed for b* values, which correspond to sample yellowness. For whey and whey proteins with lecithin, b* values were 7.37 and 12.74, while adsorption of polyphenols decreased these values to –0.31 and –0.09 for the corresponding complexes. °h values for whey and whey proteins with lecithin were 89.26 and 97.01, respectively, and increased to 359 due to adsorption of polyphenols. While adsorption of polyphenols did not change the C* value for whey proteins with lecithin (approximately 12.5), the C* value for whey proteins increased from 7.37 to 17.88.



Fig. 3. Photography of investigated complexes. The first row presents W (whey proteins) and WL (whey proteins in combination with lecithin). The second row presents W_C (complexes based on whey proteins enriched with chokeberry juice polyphenols) and WL_C (whey proteins in combination with lecithin enriched with chokeberry juice polyphenols).

3.3 IR Spectral Changes of Protein Matrices Upon Adsorption of Polyphenols

For protein structure, several regions are defined as follows: amide A (linked to N–H stretching) in the region 3300–3500 cm^{-1}, amide B (linked to N–H and C–H stretching) in the region 3100–2980 cm^{-1}, amide I (linked to C=O stretching) in the region 1700–1600 cm^{-1}, amide II (linked to N–H bending coupled with C–N stretching) in the region 1600–1500 cm^{-1}, and amide III (linked to N–H in-plane bending accompanied by C–N stretching) in the re-

Table 4. Color parameters of proteins and formulated complexes.

	L*	a*	b*	°h	C*
WL	90.50 ± 0.11^{b}	-1.57 ± 0.03^{d}	12.74 ± 0.06^{a}	97.01 ± 0.12^{c}	12.84 ± 0.06^{b}
WL_C	42.60 ± 0.11^{d}	12.34 ± 0.05^{b}	-0.09 ± 0.01^{c}	359.60 ± 0.05^{a}	12.34 ± 0.05^{b}
W	94.01 ± 0.01^{a}	0.10 ± 0.02^{c}	7.37 ± 0.04^{b}	89.26 ± 0.15^{b}	7.37 ± 0.04^{c}
W_C	52.66 ± 0.33^{c}	17.87 ± 0.06^{a}	-0.31 ± 0.04^{d}	359.00 ± 0.10^{a}	17.88 ± 0.07^{a}

WL_C – complexes based on whey proteins in combination with lecithin enriched with chokeberry juice polyphenols; W_C – complexes based on whey proteins enriched with chokeberry juice polyphenols. Different letters (a, b, c and d) represent values that are statistically different.

gion 1380–1200 cm^{-1} [28,29,30,31,32]. IR spectra of carriers and complexes enriched with chokeberry juice polyphenols, as well as their second derivatives, are presented in Figs. 4,5,6. Adsorption of chokeberry juice polyphenols onto whey proteins and onto whey proteins with lecithin decreases band intensities in the amide A and amide B regions (Figs. 4,5). A significant decrease in band intensity in the amide I and amide II regions in both protein matrices after adsorption of juice polyphenols was also recorded. The following changes were detected in the bands at 1440 cm^{-1} (linked to asymmetric CH_3 bending of the methyl groups of proteins) and 1386 cm^{-1} (linked to stretching of C–O, deformation of N–H, and deformation of C–H). When comparing whey proteins and whey proteins with lecithin, it is evident that the band at 1386 cm^{-1} is lower in intensity than the band at 1440 cm^{-1} in whey proteins, whereas the reverse ratio between these two bands was observed in whey proteins with lecithin. In addition to the decrease in band intensity, adsorption of juice polyphenols caused further changes. In whey proteins, the band at 1386 cm^{-1} was less pronounced after juice polyphenol adsorption, whereas in whey proteins with lecithin, both bands had the same intensity. There was also a change in the amide III region in both protein matrices. A band at 1222 cm^{-1} in the protein matrices became broader after adsorption of polyphenols, with a shoulder at 1271 cm^{-1}.

Additionally, the weak band at 1300 cm^{-1} disappeared. Two bands were observed for whey protein: one at 1151 cm^{-1} (linked to phosphodiester stretching) and another at 1125 cm^{-1} (linked to C–O stretching). Upon adsorption of juice compounds, the 1125 cm^{-1} band disappeared. Whey protein with lecithin contained only the 1151 cm^{-1} band (linked to phosphodiester stretching), whose intensity decreased after adsorption of juice polyphenols. On whey protein, a band at 1066 cm^{-1} shifted to 1051 cm^{-1} after adsorption of juice polyphenols, indicating changes in phosphate stretching modes. Notably, the whey protein complex with lecithin showed a band at 1103 cm^{-1} linked to symmetric P–O–C stretching. After adsorption of juice compounds, this band was overlapped by a higher-intensity band at 1047 cm^{-1}. Another band on whey protein linked to phosphodiester stretching was detected at 935 cm^{-1}; however, this band disappeared after adsorption of juice compounds. On whey protein with lecithin, this band appeared at 905 cm^{-1} with very low intensity and also disappeared after adsorption of juice polyphenols. Additional bands formed on both protein matrices after adsorption of juice polyphenols in the region of 900–600 cm^{-1}, indicating changes in CH out-of-plane bending vibrations.

Moreover, Fig. 6 compares the IR spectra of both carriers and shows clear structural differences. Several distinctions were observed, including differences in amide B (associated with N–H and C–H stretching) in the region 3100–2980 cm^{-1}, reflecting differences in carrier hydrophobicity. Differences in band intensities were also observed in the amide I, amide II, and amide III regions, resulting from lecithin bound to the whey protein structure. Another important difference appears in the 1300–1000 cm^{-1} region, corresponding to phosphate vibrations, which also clearly distinguishes the two carriers.

4. Discussion

The high number of hydroxyl groups on polyphenols provides numerous sites for interaction with proteins, forming complexes that lead to structural, functional, and nutritional changes in both proteins and polyphenols [33,34,35,36]. In general, the structures and properties of polyphenols and proteins, as well as other present compounds, govern their interactions, resulting in higher or lower binding of polyphenols to selected carriers. The predominant fraction in whey proteins is β-lactoglobulin, which contains a hydrophobic pocket situated in the internal part of the calyx structure. This region serves as the main binding site for hydrophobic and amphiphilic ligands, including polyphenols [33,37,38,39,40]. Polyphenols and β-lactoglobulin can interact via covalent and non-covalent binding, depending on the mode of complexation. The most important non-covalent interactions, after hydrogen bonding, include ionic and hydrophobic interactions [41,42,43]. Kanakis et al. [44] observed that the binding of tea polyphenols to milk β-lactoglobulin was governed by the number of hydroxyl groups that are constituent parts of the structure of the polyphenols, with binding increasing with the number of hydroxyl groups, in the following order: (−)-epigallocatechin gallate > (−)-epicatechin gallate > (−)-epicatechin > (+)-catechin (C).

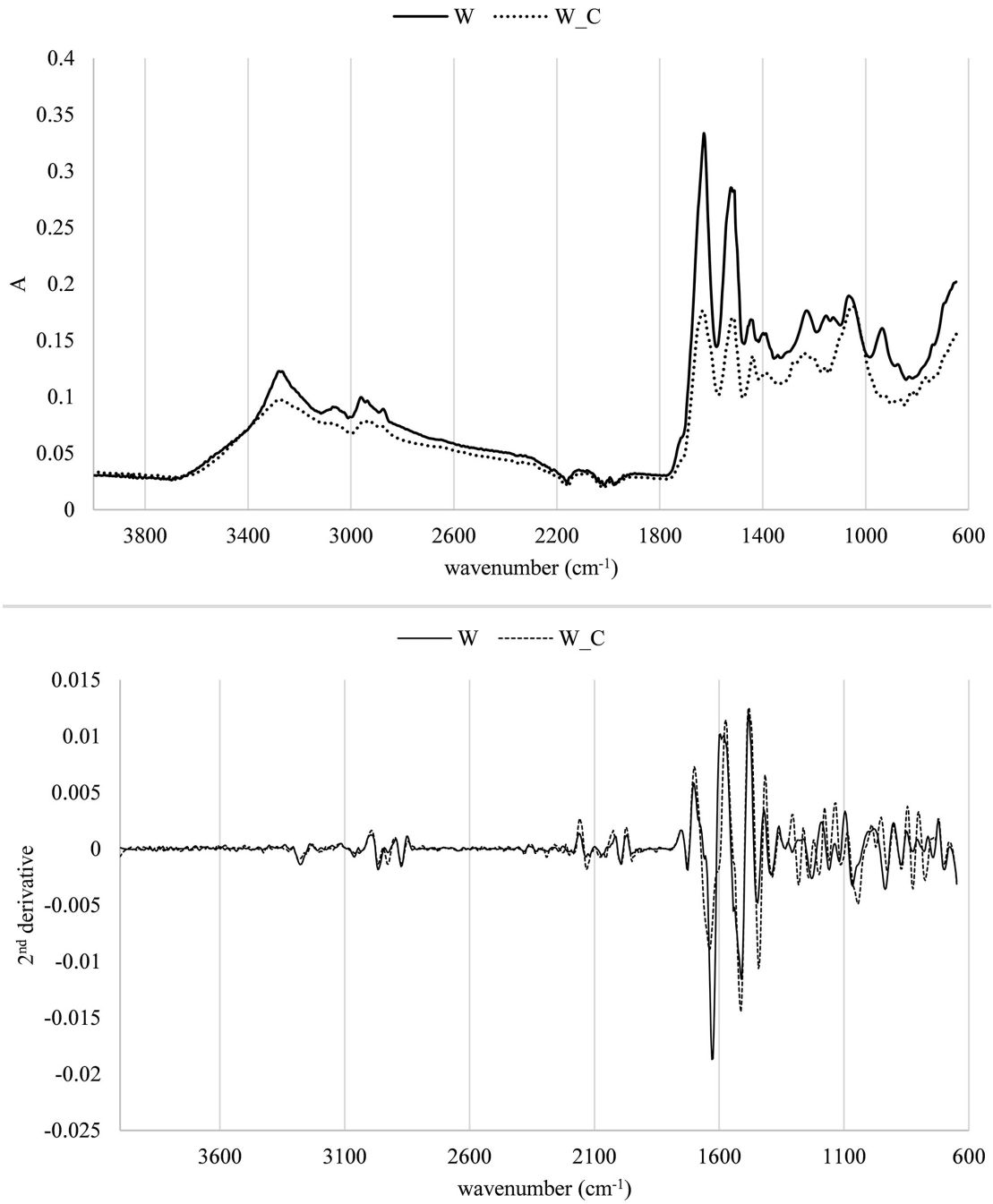


Fig. 4. Comparison of IR spectra and second-derivative IR spectra of W (whey proteins) with W_C (complexes based on whey proteins enriched with chokeberry juice polyphenols).

Comparison of polyphenol adsorption on soy proteins showed that gallic acid had the highest adsorption affinity, followed by chlorogenic acid and quercetin, which showed equal affinity. Myricetin, then caffeic acid, and then kaempferol showed a further decrease in affinity, while apigenin and flavone exhibited considerably lower affinity for soy proteins than the others [45]. Yuksel et al. [42] concluded that green tea flavonoids preferentially bind to the hydrophobic sites of dairy proteins compared to anthocyanins. Sęczyk et al. [46] determined that the affinity of polyphenols for adsorption on selected protein fractions, namely albumin and globulin, varied depending on the specific polyphenol and protein types, as well as on the complete composition of the polyphenol matrix. Chlorogenic and gallic acids showed the highest affinity for albumin; catechin and quercetin had lower affinity, while apigenin and ferulic acid showed the lowest affinity. However, the trend for globulin differed from that for albumin. Whereas apigenin and ferulic acid showed the same pattern as for albumin, chlorogenic acid showed the highest affinity, while

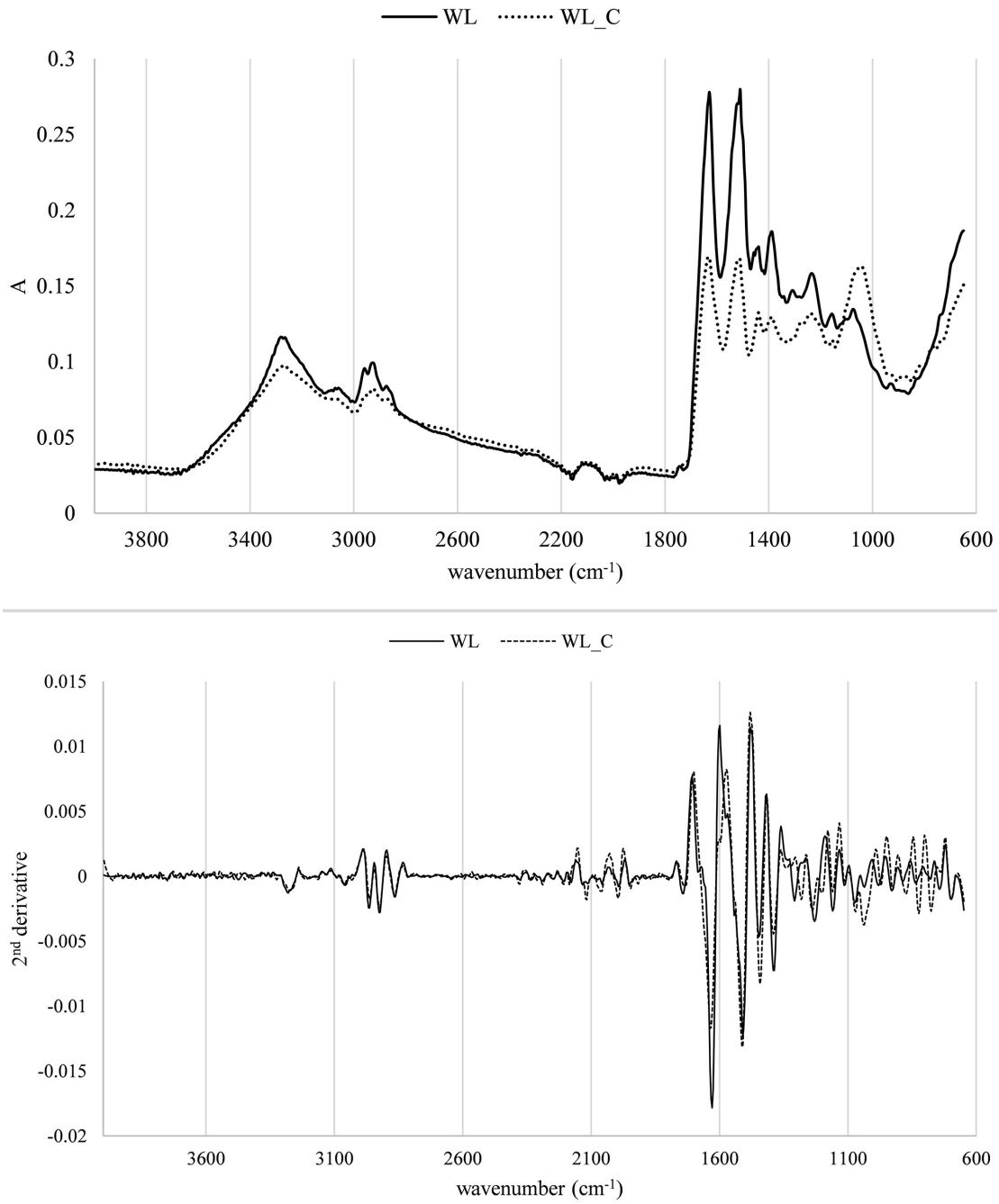


Fig. 5. Comparison of IR spectra and second-derivative IR spectra of WL (whey proteins in combination with lecithin) and WL_C (complexes based on whey proteins in combination with lecithin enriched with chokeberry juice polyphenols).

catechin and gallic acid had lower affinity than chlorogenic acid, with no difference between their affinities. Quercetin showed the lowest affinity [46].

Meanwhile, the authors investigated whether adsorption of pure polyphenolic compounds differed from that of green tea and green coffee extracts containing mixtures of compounds. The authors found that the polyphenol profile strongly affected polyphenol adsorption, so chlorogenic acid and catechin, when part of an extract, adsorbed to a greater extent than in their pure forms. Consequently, other polyphenols likely competed for available binding sites on proteins [46]. Other compounds, such as polysaccharides and lipids, can be part of protein matrices that significantly affect the affinity of polyphenols for proteins; however, polyphenols can also adsorb onto these compounds. Moreover, polyphenols can simultaneously adsorb to these additional compounds or not interact with them. Research on the binding of cranberry polyphenols to various protein matrices with different protein contents (namely defatted soybean flour with 50% protein, medium-roasted peanut flour with 50% protein, soy protein isolate with 70% protein, hemp protein isolate with 70% protein, and pea pro-

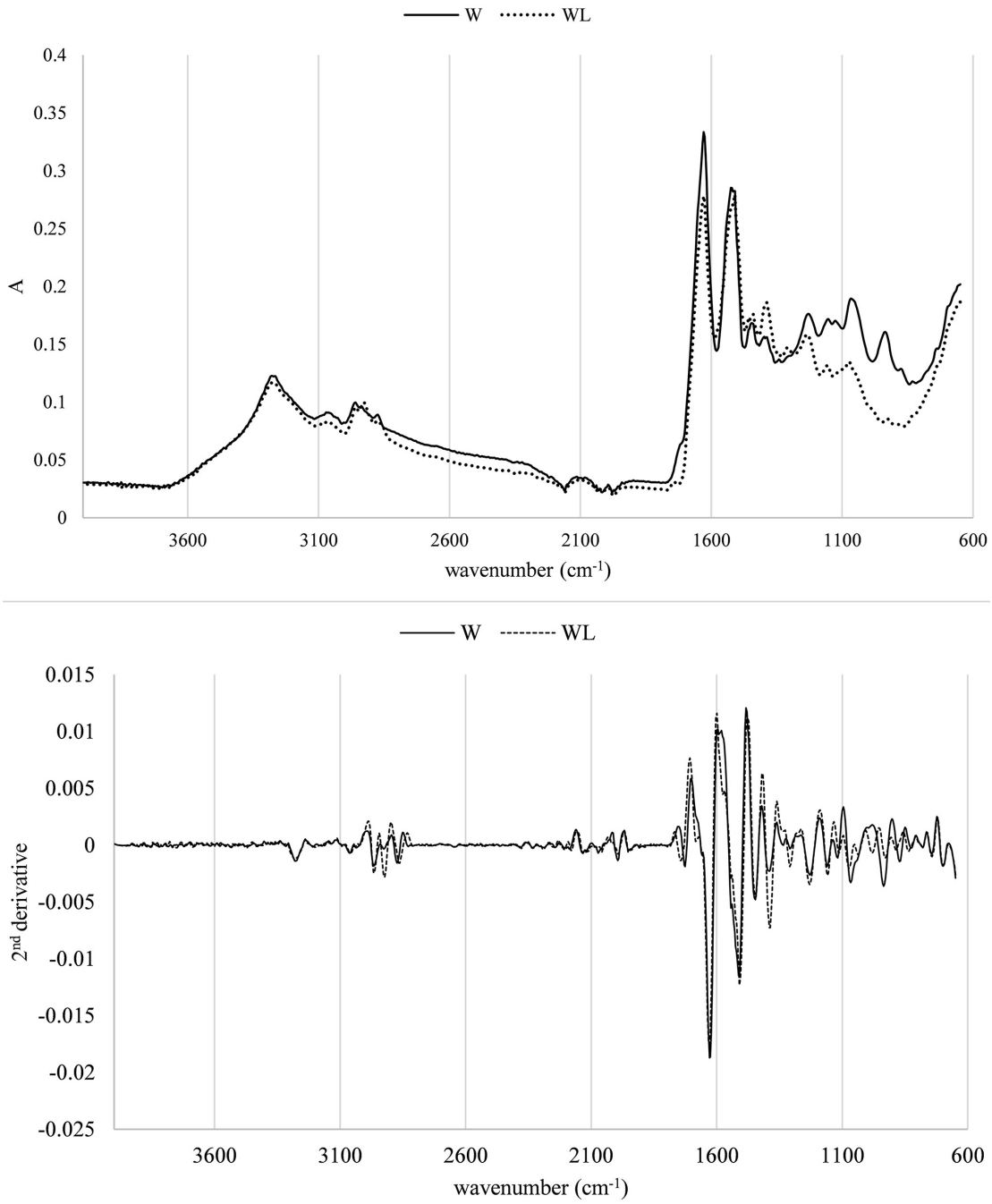


Fig. 6. Comparison of IR spectra and second-derivative IR spectra of W (whey proteins) and WL (whey proteins in combination with lecithin).

tein isolate with 85% protein) found that the highest adsorption of total polyphenols occurred on hemp protein isolate; meanwhile, the lowest was observed on soy protein isolate. Considering only anthocyanins, adsorption on soy protein isolate was again the lowest, as with total polyphenols, whereas defatted soy flour showed the highest adsorption [47]. In a study investigating the affinity of blueberry polyphenols for defatted soy flour combined with brown rice, whole wheat, or corn flour, which, in addition to proteins, contained significant amounts of polysaccharides, the authors showed that proteins and all components contributed to the adsorption of polyphenols. Comparing matrices, defatted soy flour, which had the highest protein content, also had the highest affinity for adsorbing blueberry anthocyanins. In contrast, for the other matrices, affinity decreased in the following order: whole wheat flour, brown rice flour, and corn flour [48].

Previous studies have shown that lecithin, used in various encapsulation techniques, can enhance polyphenol binding, especially for anthocyanins, through amphiphilic self-assembly that creates a segregated hydrophobic/hydrophilic microenvironment favorable for binding [19,49].

Since we used a combination of whey proteins with lecithin, and the resulting complexes had lower polyphenol content and antioxidant activity, lecithin likely occupied free polyphenol-binding sites on whey proteins. Thus, in this encapsulation technique, the positive impact of lecithin on anthocyanins, as well as on other polyphenols, was not observed. Conversely, this technique reduced polyphenol adsorption, thereby decreasing the antioxidant activity of these microparticles. The interaction between soybean lecithin and whey proteins involves hydrophobic interactions and hydrogen bonding, which alter whey protein secondary structure [50,51]. In our study, the carriers differed in structure, and these differences likely affected polyphenol adsorption onto the carriers. However, these changes reduced the adsorption of chokeberry polyphenols onto whey proteins in combination with lecithin.

The complexation mixtures contained chokeberry juice and whey protein-based matrices, meaning that a high amount of water was present due to the juice. This can also affect interactions between the protein matrices and polyphenols. The availability of water molecules in the complexation mixtures, as well as during freeze-drying, provides an environment for diffusion-related reactions. In this environment, degradation and alterations in the molecular structures of sensitive compounds such as polyphenols are linked to increased molecular mobility and oxidation reactions. As mentioned, anthocyanins are particularly sensitive to water; thus, the first step in their degradation is hydrolysis of the glycosidic bond, forming anthocyanidins, which are even more unstable than their parent molecules. More frequently, the cleavage of the pyrilium ring constitutes the subsequent step of this reaction, resulting in the formation of brown-colored chalcones [52]. However, these anthocyanin degradation products can also interact with protein matrices. In addition to the interactions between whey proteins and polyphenols and the explained impact of water, the so-called stacking effect cannot be neglected, meaning that interactions between anthocyanins from chokeberry juice and those already bound to protein can take place [53,54,55]. Therefore, it is likely that lecithin plays a substantial role not only in the interaction between whey proteins and polyphenols but also in the stacking effect. The present lecithin is probably bound to whey proteins in such a way as to block binding sites for polyphenols or cause steric hindrance. Thus, overall adsorption is affected by several different factors.

Limitations

Even though this study proved that whey complexes can be successfully formulated, assessments of storage stability and temperature stability should be conducted to provide insight into possible changes prior to incorporation into products, as well as their behavior during incorporation. This type of study should be followed by research on incorporation into food products and its impact on their quality characteristics, including controlled release of the compounds. Experiments on *in vitro* digestion and bioaccessibility could also be valuable for predicting the behavior of these compounds in the human body. However, the results of this study provide a solid basis for developing complexes based on whey and chokeberry juice polyphenols, and this approach can be extended to other polyphenol sources.

5. Conclusions

Encapsulation is one of the most relevant tools for preserving polyphenols and ensuring their stability. However, for its successful application, the choice of an adequate carrier plays an important role, and current trends favor carriers with potential health benefits. In this research, we combined chokeberry juice polyphenols with whey proteins to obtain complexes that can be used as functional food additives and additionally have potential health benefits, which can subsequently be used to enrich food products with both of these compounds. However, the choice of whey proteins in combination with lecithin proved suboptimal, emphasizing the importance of careful carrier selection. These complexes can be incorporated into food products to enhance their antioxidant potential or to enrich those products with both types of compounds.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

AP and MK designed the research study. MK, IB, DP, and AP performed the research. MK, DP, IB and AP analyzed the data. MK, IB and AP wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflicts of Interest

Dubravko Pichler is employed by Water Supply-Osijek. This employment is unrelated to the subject matter of this study. The company had no involvement in the study

design, data collection, analysis, interpretation, manuscript preparation, or decision to publish.

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