biomass

MDPI

Article

Isolation of Polyphenols from Two Waste Streams of Clingstone Peach Canneries Utilizing the Cloud Point Extraction Method

Ioannis Giovanoudis [1,2], Vassilis Athanasiadis [1], Theodoros Chatzimitakos [1], Dimitrios Kalompatsios [1], Eleni Bozinou [1], Olga Gortzi [2], George D. Nanos [2] and Stavros I. Lalas [1,*]

[1] Department of Food Science and Nutrition, University of Thessaly, Terma N. Temponera Street, 43100 Karditsa, Greece; gio@uth.gr (I.G.); vaathanasiadis@uth.gr (V.A.); tchatzimitakos@uth.gr (T.C.); dkalompatsios@uth.gr (D.K.); empozinou@uth.gr (E.B.)

[2] Department of Agriculture Crop Production and Rural Environment, School of Agricultural Sciences, University of Thessaly, 38446 Volos, Greece; olgagortzi@uth.gr (O.G.); gnanos@uth.gr (G.D.N.)

* Correspondence: slalas@uth.gr; Tel.: +30-24410-64783

Abstract: This study aimed to assess the feasibility of employing cloud point extraction (CPE) as an efficient way of extracting polyphenols from peach waste (PW). Four distinct food-grade surfactants (Genapol X-080, PEG 8000, Tween 80, and lecithin) were evaluated at concentrations ranging from 2–10% *w*/*v* to determine the efficiency of the technique in two separate PW streams [i.e., lye peeling waste stream (LPWS) and total wastewater stream (TWS)]. Low amounts (2% *w*/*v*) of surfactants in a single-step CPE were found to result in less than ~61% polyphenol recovery in LPWS and less than ~69% polyphenol recovery in the TWS, necessitating additional extraction steps. In both PW streams, the single-step polyphenol recovery was improved by 25–67% utilizing a higher amount of surfactants (5–10% *w*/*w*), leading to a statistically significant figure ($p < 0.05$). The CPE procedure was conducted under optimal conditions, including a temperature of 65 °C, a sodium chloride concentration of 3% *w*/*v*, a pH level of 3.5, and a surfactant concentration of 5% *w*/*v*. The polyphenol recovery was efficient when the CPE procedure was conducted twice. Tween 80 proved to be the most efficient surfactant among the four tested surfactants, achieving recoveries above 98% in both PW streams. Under optimum extraction conditions, the total polyphenol content and antiradical activity of PW extracts were evaluated. The results showed statistically significant differences ($p < 0.05$) between the two PW streams, with the LPWS having approximately 12 times higher polyphenol content and being more potent, achieving ~64% antiradical activity. Using the LPWS instead of the TWS is a more cost-effective and feasible option for the industry. In addition, the considerable volume of the TWS makes it challenging to handle and demands a correspondingly major amount of surfactant. Considering that Tween 80 is a low-toxicity surfactant and that the CPE method is simple, fast, cost-effective, highly accurate, and selective, the extracted polyphenols from two PW streams could be exploited as natural antioxidants to be used directly in the food industry. These findings could have major implications for the manufacturing of sustainable and naturally-derived food additives.

Keywords: food-grade surfactants; lye peeling waste stream; total wastewater stream; natural antioxidant recovery; food industry

Citation: Giovanoudis, I.; Athanasiadis, V.; Chatzimitakos, T.; Kalompatsios, D.; Bozinou, E.; Gortzi, O.; Nanos, G.D.; Lalas, S.I. Isolation of Polyphenols from Two Waste Streams of Clingstone Peach Canneries Utilizing the Cloud Point Extraction Method. *Biomass* **2023**, *3*, 291–305. https://doi.org/10.3390/biomass3030018

Academic Editor: Lasse Rosendahl

Received: 29 June 2023
Revised: 5 August 2023
Accepted: 23 August 2023
Published: 4 September 2023

1. Introduction

The food industry is responsible for a significant portion of the overall amount of wasted food. Such waste is composed of, primarily, organic residues from the processing of raw materials. The generation of waste is an inevitable outcome of the production process. However, managing product-specific waste poses significant challenges due to factors such as insufficient biological stability, potential pathogenicity, high content, susceptibility to rapid autoxidation, and elevated enzymatic activity [1]. Inefficient management of waste results in discharges that are major contributors to environmental pollution [2]. The

food industry generates waste with high chemical oxygen demand due to its high organic matter and nutritional content, which poses a serious environmental concern [3]. One sector that generates substantial waste is the fruit and vegetable processing industry. This kind of food waste contains several valuable bioactive substances, including proteins, polysaccharides, pigments, polyphenols (and flavonoids), and dietary fiber, distinguishing it from other industrial waste [4]. The bioactive compounds present in food waste could be valorized so as to be used as food additives. Additionally, they can be transformed into more complex chemicals, macromolecules, or biofuels for multiple uses [5]. The failure to utilize food waste results in ecological risks, nutrient loss, and economic disadvantages. De Leonardis et al. [6] have highlighted the potential of these compounds for the development of innovative food products.

Various technologies, including membrane-based technologies, ozonation, adsorption, and solvent extraction, have demonstrated their effectiveness in decreasing the chemical oxygen demand, soluble solids, and other impurities in food waste [7,8]. However, most of these techniques are unsuitable for fruit waste owing to their elevated chemical oxygen demand, biomacromolecule content, and viscosity. Microbial digestion, including both aerobic and anaerobic approaches, is frequently employed in wastewater treatment processes. While these procedures are generally effective, they are capable of raising the detrimental microbes and lead to high levels of biological oxygen demand [9]. Recent advances in liquid–liquid extraction techniques, particularly those incorporating multiphase separation, have attracted increased attention for their efficiency in extracting and concentrating bioactive compounds from natural sources. Novel methodologies such as aqueous two-phase extraction, dispersive liquid–liquid extraction, micellar extraction, and cloud-point extraction (CPE) have been developed [10,11]. However, liquid–liquid extraction techniques have limitations, including emulsion formation, use of harmful organic solvents, and generation of pollutants, despite their potential benefits. These factors make the process laborious, costly, and ecologically unsustainable [12]. Consequently, there is an immense incentive to develop methods and technologies for nutrient recovery and reuse of food waste [13]. The utilization of CPE is a sustainable approach for the extraction of bioactive compounds derived from plant sources [14]. This kind of extraction has the potential to be applied in various industries, such as pharmaceuticals and food industries. The CPE method is a cost-effective and straightforward approach to extract bioactive compounds from liquid matrices with the use of surfactants [15]. Briefly, the procedure involves the addition of a surfactant to a liquid sample along with a salt, the maintenance of cloud point temperature, centrifugation, and finally separation from the liquid sample. This is a one-step extraction that can be repeated for more efficient bioactive compound recovery [16]. Food-grade surfactants can be used to extract the target compounds and consequently, incorporate these compounds directly into food products [17]. Micelles are formed at a critical concentration in aqueous solutions, which are in dynamic equilibrium with the bulk aqueous solution monomers [18]. Separation can be accomplished by binding hydrophobic and hydrophilic molecules to these structures through dipole–dipole interactions and hydrogen bonding [19]. Several surfactants, namely, Triton X-100, Triton X-114, and Span 80 have been successfully utilized to isolate bioactive compounds [16].

Polyphenols are prevalent in most fruits and vegetables and can also be found in fruit and vegetable by-products. These compounds exhibit antioxidant and cytoprotective properties, highlighting their potential health advantages in human nutrition [20]. Plants employ polyphenols to maintain antioxidant or antimicrobial functions as a way of protection. They shield plants from UV light, parasites, and insects and are responsible for the plants' pigment and astringency. A wide variety of plant sources, including fruits and vegetables, contain such compounds [21]. Peach, also known as *Prunus persica*, is a type of climacteric stone fruit that has its origins in China. Through the years, it has become widely distributed in various parts of the world, including the USA, Spain, Italy, and Greece [22]. The high levels of polyphenols, vitamin C, carotenoids, and fiber it contains are the reason that this product has excellent nutritional value. Antioxidant, anti-inflammatory,

and anticarcinogenic effects are just a few of the many positive health effects associated with these compounds [23]. The peach industries release an immense quantity of waste, including peels and wastewater (which usually derives from solid peach waste), which are frequently disposed of in landfills or surface waters. Peach peels contain two– three times more polyphenols and carotenoids than fruit flesh, according to a study by Chang et al. [24]. Food-related waste streams are indeed a substantial source of secondary metabolites with bioactive properties, especially polyphenols [25]. Peels are common b-products of food processing that contain a significant amount of polyphenols [26].

To the best of our knowledge, investigations on polyphenol extraction from peach cannery waste utilizing CPE are sparse. While there is a growing body of research on the extraction of bioactive compounds from various food waste sources, there is a lack of specific studies focusing on polyphenol extraction from peach cannery waste. This is particularly noteworthy considering the significant volume of waste generated by the peach processing industry. The purpose of this research was to investigate the potential of CPE, employing non-hazardous, food-grade surfactants (i.e., Genapol X-080, PEG 8000, Tween 80, and lecithin) for polyphenol extraction from peach cannery waste streams. Two different peach waste (PW) streams were studied, i.e., waste coming from the lye peeling wastewater stream (LPWS) and total wastewater (TWS) from all activities of processing were used. The objective of this study was to develop a comprehensive method for effectively controlling and maximizing the value of the produced waste by the peach processing industries. The surfactants with their concentrations, along with multiple steps of CPE, were assessed. The study also included the assessment of the overall polyphenol content and antiradical activity of the polyphenols isolated from the two PW streams.

2. Materials and Methods

2.1. Chemicals, Reagents, and Materials

Genapol X-080, methanol, and DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) were purchased from Sigma–Aldrich (Steinheim, Germany). Folin–Ciocalteu reagent, gallic acid, and anhydrous sodium carbonate were bought from Penta (Prague, Czech Republic). PEG 8000 was from Alfa Aesar (Karlsruhe, Germany). Citric acid anhydrous was obtained from Merck (Darmstadt, Germany). Tween 80 was bought from Panreac (Barcelona, Spain). Soya lecithin (>97%) and sodium chloride were both obtained from Carlo Erba (Milano, Italy). A deionizing column was employed to generate the deionized water used in the experiments.

Two streams, i.e., LPWS and TWS were obtained from ELBAK S.A. (Falani, Larissa, Greece) during the processing of clingstone peaches (*Prunus persica*) of the Everts variety.

2.2. Peach Waste Streams

Figure 1 shows a typical clingstone peach processing flowchart. Canning uses styles such as halves (mainly) and also slices and cubes. All sound fruit rejections (e.g., broken fruit pieces, soft fruit), but also other sound fruit (e.g., too large, too small, or just to add to the total capacity of the puree line) are driven to the aseptic puree line with an option of concentration using a typical double-stage evaporator. The initial stage of the manufacturing process involves the cleansing of the peaches. Following the washing process, the peaches are subsequently transported along a belt conveyor for the purpose of sorting, whereby the peaches of superior quality are directed towards the pitters (machines for stone removal). The pitters also cut the fruit into two halves and the peeling takes place. It is there that the LPWS can be derived. Most halves are used for canning, but fruit pieces sorted out are driven to the puree line. For this line, crushing follows and then blanching. To prevent enzymatic browning, a common concern in fruits, the process of blanching involves subjecting crushed fruits to rapid heating at temperatures ranging from 90 to 95 °C. This elevated temperature is maintained for a duration of approximately 2 to 3 min. The reason for this phenomenon is that heat causes the deactivation of the enzymes that are accountable for the darkening process, while also inducing a softening effect on the fruit, thereby facilitating subsequent processing procedures [27]. This heating

process is typically conducted in a tubular heat exchanger. After the heating process, the crushed fruit is delivered to the refiner, i.e., driven through rotated sieves where puree is produced, and to the deaerator. Dry matter content of 25–30% (typically 28% or 36% minimum) is attained via transporting dense and viscous peach puree concentrate (PPC) to the evaporation system. The aseptic packaging unit collects PPC from the evaporator and places it in barrels for later use. Before the filling heads in a commercial aseptic line, a second tubular heat exchanger is used for cooling and sterilization. Two hours was settled on as the acceptable time for PPC creation. The figure shows that the LPWS occurs following the peach peeling stage, as mentioned before. It is also highlighted that during different stages of peach processing, a wastewater stream is generated. These streams are mixed with other liquids to form the TWS stream. It could be deduced that the TWS includes a quantity of the LPWS stream diluted with liquids from various treatment stages. It is also reasonable to assume that the total volume of the TWS stream is significantly larger than that of the LPWS.

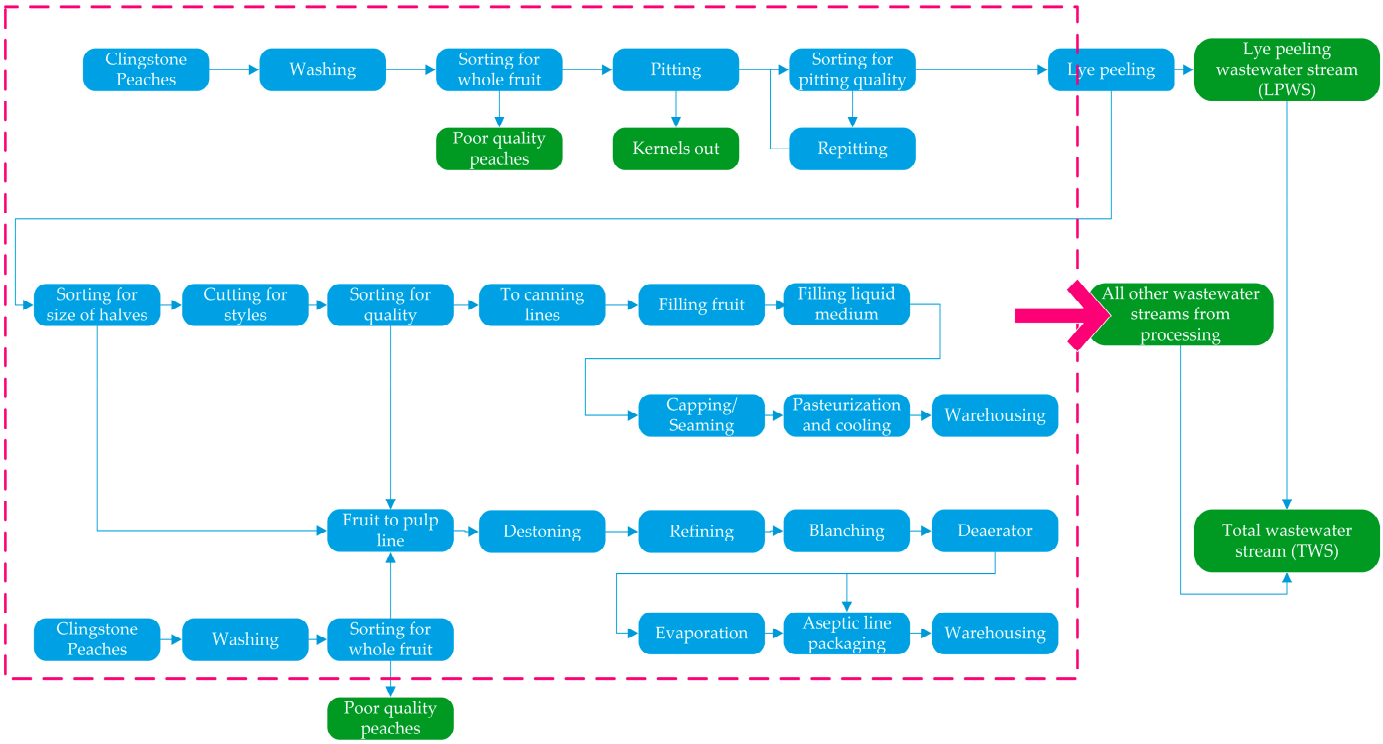

Figure 1. A typical clingstone peach processing flowchart.

2.3. CPE Procedure

The PW was removed from the refrigerator and thermostated at room temperature (~25 °C) for an hour. A Remi Neya 16R (Remi Elektrotechnik Ltd., Palghar, India) was utilized to centrifuge PW for 20 min at 4500 rpm and at 30 °C, to remove the solids. Solid-free PW samples were acidified to a pH of 3.5 using 0.66 M citric acid before being treated with CPE [28]. Preliminary tests were used to select the experimental conditions (i.e., pH, sodium chloride concentration, temperature, etc.). To accelerate the phase-separating process, sodium chloride was added to the sample to increase the bulk density of the aqueous phase. The temperature at which clouds form is also lowered by sodium chloride [29].

The CPE technique was implemented as described by Chatzilazarou et al. [30]. A quantity of 50 g of PW was mixed with 3% *w*/*v* sodium chloride and 2–10% *w*/*v* of each surfactant. The mixture was heated to a constant temperature and stirred using a magnetic stirrer (Heidolph MR Hei-Standard, Schwabach, Germany). For 20 min, the samples were equilibrated at 65 °C while being stirred at 800 rpm. After centrifuging the mixture for 5 min at 3500 rpm at 30 °C, the phases were decanted (first extraction step), while the surfactant phase was viscous. After

centrifugation, the volumes of surfactant and water were measured. Then, the water phase was decanted and the above procedure was repeated either once (as the second extraction step) or twice (as the third extraction step), in order to maximize the polyphenol recovery. Each CPE experiment was repeated three times under identical conditions; hence, all recovery values reflect the means of three extraction experiments.

2.4. Polyphenol Recovery by CPE

The calculation of polyphenol recovery was performed using a polyphenol mass balance. The estimation of surfactant recovery was conducted in accordance with prior methodologies [30–32]:

$$\text{Recovery } (\%) = \frac{Cs \cdot Vs}{Co \cdot Vo} \times 100 = Co \cdot Vo - \frac{Cw \cdot Vw}{Co \cdot Vo} \times 100 \quad (1)$$

where Cs is the polyphenol concentration in the volume Vs of the surfactant phase, Co is the polyphenol concentration in the volume Vo (10 mL) of the initial sample, and Cw is the polyphenol concentration in the volume Vw of the water phase. The concentration of each phase was calculated with a Folin–Ciocalteu method (*vide infra*) as mg GAE/L of PW.

2.5. Determination of Total Polyphenol Content

Total polyphenol content (TPC) was measured photometrically using a modified Folin–Ciocalteu method by Chatzilazarou et al. [30]. Briefly, 100 µL of the sample was mixed with 100 µL of the Folin–Ciocalteu reagent, and after 2 min, 800 µL of sodium carbonate solution (5% w/v) was added. The absorbance of the solution was then measured at 750 nm using a Shimadzu spectrophotometer (UV-1700, Shimadzu Europa GmbH, Duisburg, Germany) after 20 min incubation at 40 °C in the absence of light. The results were expressed as mg GAE/L of PW.

2.6. Determination of Antiradical Activity

The antiradical activity (A_{AR}) of recovered polyphenols in the surfactant phase after CPE treatment was determined using the DPPH$^{\bullet}$ method as established by Tsaknis and Lalas [33], with some modifications regarding the formula used. In brief, 4 mL of the sample were mixed with 1 mL of 0.1 mM DPPH$^{\bullet}$ solution in methanol. The mixture was homogenized and incubated in the absence of light for 30 min at ambient temperature. Absorbance was photometrically measured at 517 nm. A control sample including DPPH$^{\bullet}$ solution and methanol instead of sample was also used and the absorbance was measured immediately. The % scavenging was calculated using the given equation:

$$\%\ \text{Scavenging} = A_{\text{control}} - \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (2)$$

where A_{control} and A_{sample} denote the respective absorbances.

2.7. Statistical Analysis

Each analysis was done three times. Results were presented as means of three replicates ± standard deviation. Statistically significant differences were tested using the Kruskal–Wallis test, after testing the data using the Kolmogorov–Smirnov test. Statistically significant differences were considered at $p < 0.05$.

3. Results and Discussion

The study evaluated the efficiency of four different food-grade surfactants, namely, Genapol X-080, PEG 8000, Tween 80, and lecithin, in terms of their ability to facilitate polyphenol extraction. Based on the results of preliminary experiments, we chose the parameters to improve the extraction of polyphenols. The CPE procedure was carried out at 65 °C, with a 3% w/v concentration of sodium chloride, and a pH of 3.5 to assure

consistency. According to prior research conducted by Kiai et al. [34], it was found that the optimum temperature range for CPE is between 50–70 °C, particularly when utilizing surfactants such as Genapol X-080, Tween 80, and Triton-X. The optimal pH level was determined to be 3.5 due to the protonation of polyphenols at low pH values. This results in strong interaction with micellar aggregates of non-ionic surfactants, thereby increasing their solubility in the micelle [35]. At higher pH levels, polyphenols undergo deprotonation, resulting in decreased solubility within hydrophobic micelles [36]. The investigation examined the recovery of polyphenols through testing three different concentrations 2, 5, and 10% w/v of surfactants, and employing three successive extraction procedures. It is important to note that the concentration of the surfactant has a key role in the efficiency of the extraction of bioactive compounds. The preconcentration factor decreases as the surfactant final volume increases, lowering the analytical signal. On the other hand, lowering surfactant concentration would likely reduce accuracy and reproducibility since the surfactant-rich phase would not be enough to conduct reproducible extraction and separation measurements [37]. As such, the optimum surfactant concentration should be thoroughly considered. As per Santana et al. [28], higher concentrations of surfactants are required to achieve higher polyphenol extraction yields. In both PW streams, the initial extraction step exhibited statistically significant variations ($p < 0.05$), confirming the expected trend that higher surfactant concentrations lead to higher extraction of polyphenols via CPE (*vide infra*).

3.1. Polyphenol Extraction with Genapol X-080

The non-ionic surfactant Genapol X-080 is an alkylpolyethylene glycol ether. Genapol X-080 has a hydrophilic polyethylene glycol (PEG) chain attached to a lipophilic alkyl chain. It is widely employed as an emulsifier, solubilizer, and wetting agent in a variety of industries, including food, cosmetics, and pharmaceuticals [38,39]. Figure 2 displays the results obtained using Genapol X-080 in the two PW streams, which show a clear relationship between the amount of surfactant used and polyphenol recovery. In LPWS, the first step of CPE with 2%, 5%, and 10% w/v of Genapol X-080 had extraction yields of 54.6%, 72.1%, and 85.5%, respectively. In the TWS, the corresponding polyphenol recovery values were higher and were measured to be 64.2%, 80.2%, and 90.3%. In both PW streams, it was found that it was cost-effective to use 2% w/v of Genapol X-080 three times since it achieved almost as much recovery as one-step extraction with 10% w/v of the surfactant. No statistically significant variations ($p > 0.05$) were found among these measurements. However, the most efficient extraction yields were observed using 5% w/v of the surfactant twice, achieving 91.2% and 93.9% in the LPWS and TWS, respectively. Our results are consistent with Chatzilazarou et al. [30], who investigated the polyphenol extraction from wine sludge using 2% Genapol X-080. The authors stated that the optimal extraction conditions were pH 3.5, temperature 55 °C, and 30 min of extraction time and they recorded 75.8% polyphenol recovery. In another study, Kiai et al. [34] used cloud point extraction with Genapol X-080 as the surfactant to preconcentrate polyphenols from table olive processing wastewaters. Optimal conditions were determined as follows: 10% w/v surfactant concentration, pH level of 2, temperature of 70 °C, and an equilibrium time of 30 min. Under optimized conditions, the polyphenol recovery yield from table olive processing wastewaters using one-step CPE was 68%. Overall, the use of Genapol X-080 as a surfactant in CPE for polyphenol extraction from PW streams has demonstrated promising results, with optimal concentrations and extraction steps leading to efficient recovery of polyphenols.

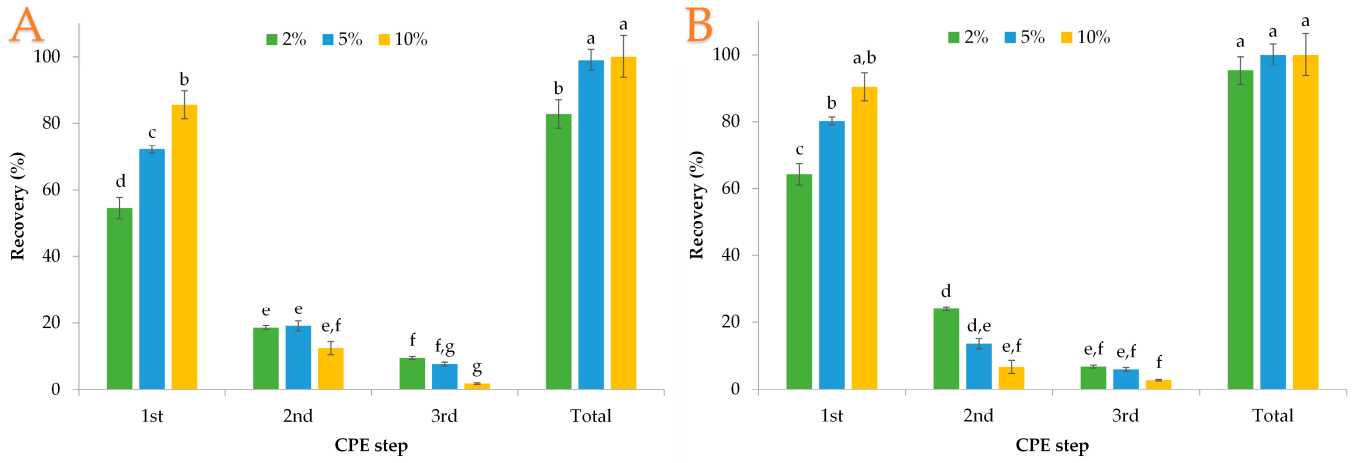

Figure 2. Recovery of polyphenols in LPWS (**A**) and TWS (**B**) using Genapol X-080 at varied concentrations and extraction steps; error bars represent the standard deviation; samples that statistically differ ($p < 0.05$) are denoted with different letters (i.e., a–g).

3.2. Polyphenol Extraction with PEG 8000

Water-soluble polyether PEG 8000 is a commonly used waxy solid that functions as a lubricant, thickening agent, solvent, and surfactant in various applications, including the food, pharmaceutical, and cosmetic industries. It is considered safe for human consumption due to its low toxicity [40]. Figure 3 presents the results of polyphenols extracted through CPE from LPWS and TWS samples, utilizing three different concentrations of PEG 8000. The figure demonstrates the strong dependence of polyphenol extraction on the surfactant concentration. Increased amounts of PEG 8000 led to increased recoveries of polyphenols. Nevertheless, the utilization of reduced concentrations of PEG 8000 (i.e., 2% w/v) resulted in a recovery below 60%, thereby emphasizing the significance of supplementary CPE steps. In LPWS, the extraction yields of the initial step for 2%, 5%, and 10% w/v PEG 8000 were 52.9%, 68.8%, and 81.5%, respectively. In the second extraction step, the extraction yields were 70.6%, 86.6%, and 91.9%. The corresponding extraction yields were slightly elevated in the TWS. In the first extraction step, the polyphenol extraction was elevated by 9.1–18.2%, and in the second extraction step, the recovery was increased by 8–18%. The most efficient concentration of PEG 8000 was found to be 5% w/v in a two-step extraction, yielding 86.6% polyphenol recovery in the LPWS and 93.4% polyphenol recovery in the TWS. In a previous study [30] focusing on the recovery of polyphenols from wine sludge with CPE, PEG 8000 was also used as a surfactant. The optimum conditions were found to be a two-step extraction utilizing 10% w/v of PEG 8000 in total and establishing pH level at 2.5, temperature 55 °C, and extraction time of 30 min. The achieved polyphenol recovery was measured to be 98.5%. Overall, the use of PEG 8000 as a surfactant in CPE for polyphenol extraction from PW streams has shown promising results. The concentration of PEG 8000 plays a crucial role in achieving efficient recovery, with the optimal concentration and extraction steps leading to high polyphenol yields.

In our previous study [41] regarding the polyphenol recovery from apricot wastewater, PEG 8000 was used in a concentration of 2% w/v in a two-step CPE procedure and resulted in efficient recovery (~99%), which was ~55 mg GAE/L. In the case of peach wastewater (TWS), the use of 2% PEG 8000 in a two-step CPE process had an overall yield of ~83%, which resulted in ~42 mg GAE/L. The difference in the percentage polyphenol recovery is most likely due to the difference in chemical composition of the two fruits, but further investigation would be of high interest.

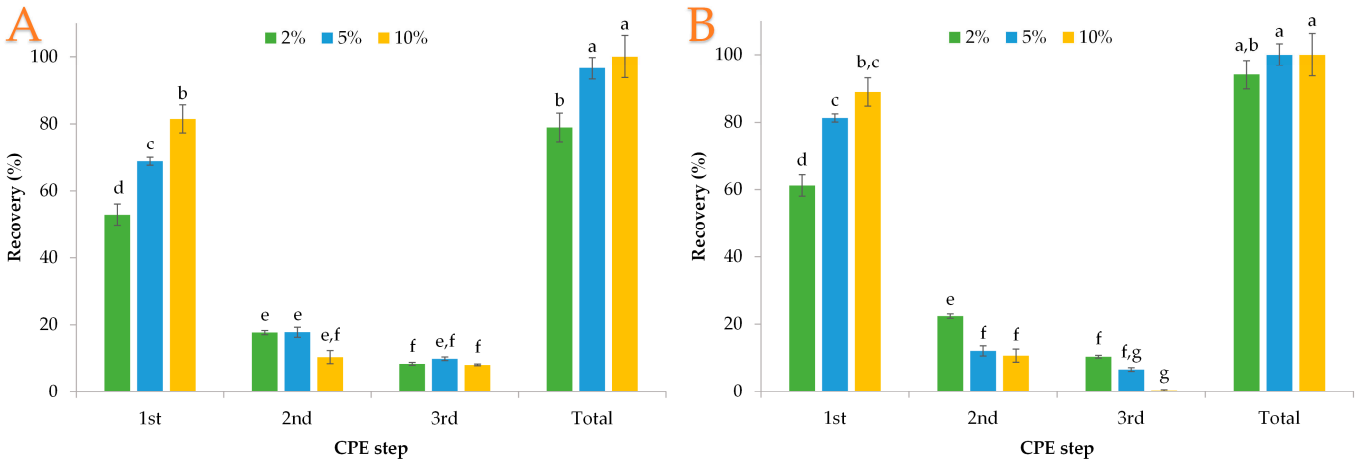

Figure 3. Recovery of polyphenols in LPWS (**A**) and TWS (**B**) using PEG 8000 at varied concentrations and extraction steps; error bars represent the standard deviation; samples that statistically differ ($p < 0.05$) are denoted with different letters (i.e., a–g).

3.3. Polyphenol Extraction with Tween 80

Polysorbate 80, also known as Tween 80, is a non-ionic surfactant within the polysorbate group. It is a water-soluble liquid that is often employed as a solubilizer, emulsifier, and stabilizer in a variety of industries such as the food and pharmaceutical industries. It derives from natural substances including ethylene oxide, sorbitol, and oleic acid. Tween 80 is recognized for its ability to enhance the solubility and bioavailability of poorly soluble pharmaceuticals, in addition to its emulsification properties in food products [42]. Given its versatility, it has plenty of applications and is recognized as safe by regulatory agencies, such as the Food and Agriculture Organization [43]. The outcomes of polyphenol extraction via CPE from LPWS and TWS samples, using three concentrations of Tween 80, are shown in Figure 4. Statistically significant differences ($p < 0.05$) were observed in the first and second extraction steps in relation to the surfactant concentration. In the LPWS, the initial CPE step using 2%, 5%, and 10% w/v of Tween 80 recorded 60.3%, 77.3%, and 90.2% recovery, respectively. The second extraction step was crucial for the recovery of a sufficient amount of polyphenols. The recorded polyphenol recovery was 81.7%, 98.8%, and 99.5%. It was shown again that the most effective surfactant concentration was 5% w/v in a two-step extraction since it achieved ~99% recovery of polyphenols, the same as using 10% surfactant in a two-step extraction. This is of high importance in the case of large amounts of PW, as it would also save large amounts of surfactant. In the TWS, even by using a single extraction step, the results were satisfactory, with recoveries ranging from 68.2–95.4%. In the second extraction step, the recovery rates were 93.4–100%. These results indicate that this surfactant had better polyphenol recoveries than the other surfactants. Two-step extraction using 5% w/v Tween 80 appears to be an economical yet effective way of extracting polyphenols from any PW stream. Our results are comparable to that of Katsoyannos et al. [44] who studied the potential use of low-biological-hazard surfactant Tween 80 in the extraction of natural antioxidant compounds (polyphenols and carotenoids) found in olive mill wastewater and red-fresh orange juice using CPE. The optimum conditions for extraction were 55 °C, 20% w/v sodium chloride, and pH 3. In the initial extraction step with Tween 80, olive mill wastewater and red-fresh orange juice recorded 86.8 and 55.4% recovery, respectively. At the second extraction step, the respective extraction yields measured 94.4 and 79.8%. Stamatopoulos et al. [45] studied the polyphenol recovery in olive leaf extracts. The optimal conditions for this process were determined to be a pH level of 2.6, an ambient temperature of 25 °C, 2% of Tween 80 (w/v) in a two-step extraction (4% w/v in total), 35% sodium sulfate (w/v), and a settling time of 5 min. The recorded extraction yield was measured at 95.9%. Overall, Tween 80 has proven to be an effective surfactant for polyphenol extraction through CPE from various

PW streams. The concentration of Tween 80 significantly influences the recovery and as such, the necessary extraction steps.

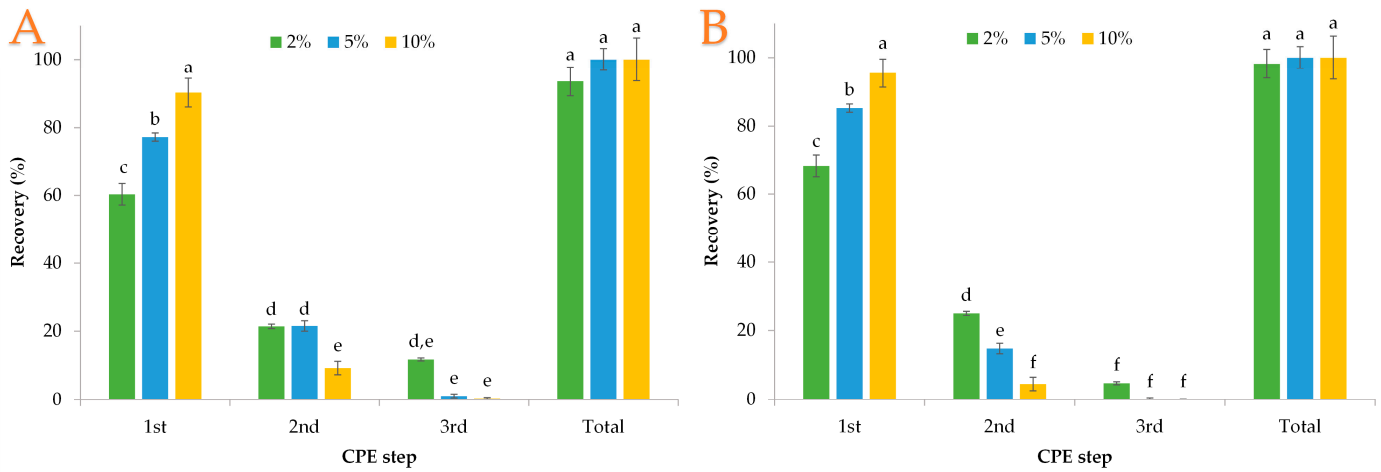

Figure 4. Recovery of polyphenols in LPWS (**A**) and TWS (**B**) using Tween 80 at varied concentrations and extraction steps; error bars represent the standard deviation; samples that statistically differ ($p < 0.05$) are denoted with different letters (i.e., a–f).

3.4. Polyphenol Extraction with Lecithin

Lecithins are amphiphilic molecules that occur naturally and consist of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid [46]. These emulsifiers are commonly utilized in the food industry and according to European Regulations regarding food additives [47], they are not subject to any maximum level limitations (quantum satis). Lecithins are a desirable substitute for synthetic ingredients due to regulatory requirements and the health advantages of phospholipids [46]. Despite the lower extraction yield per step compared to other surfactants, lecithin is a cost-effective, naturally occurring, edible, and non-hazardous surfactant alternative [48]. Figure 5 displays the outcomes of polyphenol extraction from PW samples utilizing three distinct lecithin concentrations. Statistically significant differences ($p < 0.05$) were mainly observed between the three lecithin concentrations in the first extraction step. In LPWS, the first CPE step utilizing 2, 5, and 10% w/v of Tween 80 recorded 45.8, 61.2, and 76.4% polyphenol recovery. In comparison to other surfactants, it appears that with one-step extraction, the recovery of polyphenols was poor, only about 46% for 2% w/v surfactant concentration. This highlights the need for multiple stages for more efficient extraction of polyphenols. In fact, from the very second extraction, the recovery rates increased significantly, especially in the case where 10% w/v lecithin was used. In the second extraction step, the corresponding recoveries were measured at 66, 83.5, and 99.4%. In TWS, the polyphenol recoveries measured in the first extraction step were slightly increased by 2.5–21.2%. With an additional extraction step, almost all polyphenols were recovered, since a recovery rate of 92.4–100% was achieved. The results are comparable to that of Alibade et al. [49] who studied the optimization of polyphenol recovery conditions from wine waste with the natural surfactant lecithin. The optimal conditions were found to be a surfactant concentration of 5% w/v, multiple CPE steps, a pH value of 3, and a temperature of 40 °C. In the three extraction steps with this lecithin concentration, the polyphenol recoveries were measured at 65, 79.4, and 87.4%, which are close to our values (61.2%, 83.5%, and 92.5%). Karadag et al. [50] investigated the polyphenolic enrichment of lecithin from olive mill wastewater through cloud point extraction. The optimal conditions for the process were found to be a temperature of 65 °C, pH level of 4.5, sodium chloride concentration of 10% (w/v), and lecithin concentration of 15% (w/v). The achieved polyphenol recovery in a single extraction step was measured at around 50%. In conclusion, lecithin, as a natural surfactant, demonstrates the promising potential for polyphenol extraction from various PW streams. While its extraction yield per step may be lower compared to other

surfactants, lecithin's cost-effectiveness, natural origin, and non-hazardous nature make it an attractive alternative for polyphenol extraction. Further optimization of extraction conditions can enhance its efficiency and overall effectiveness.

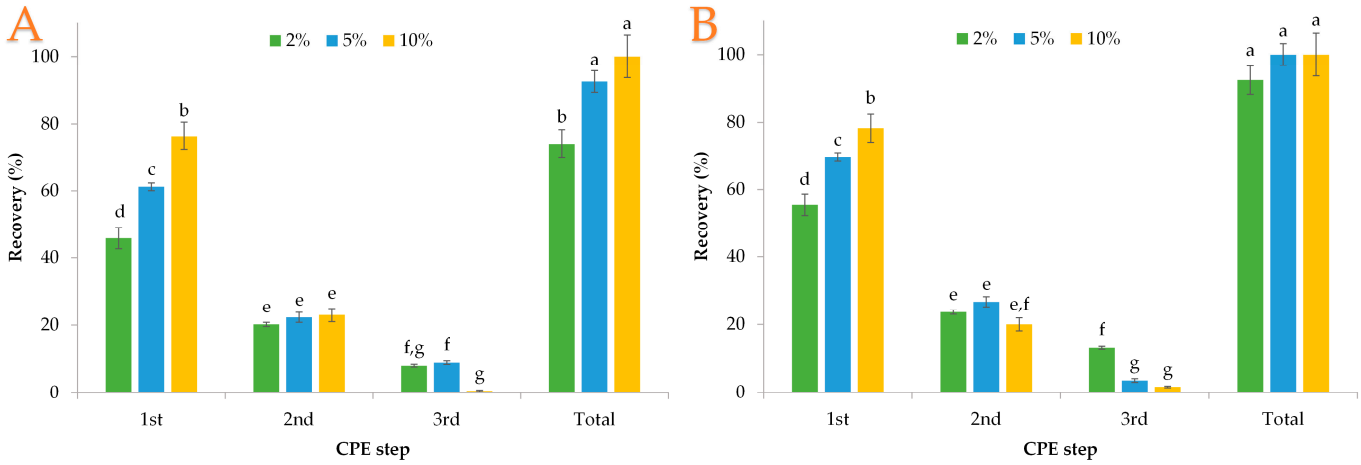

Figure 5. Recovery of polyphenols in LPWS (**A**) and TWS (**B**) using lecithin at varied concentrations and extraction steps; error bars represent the standard deviation; samples that statistically differ ($p < 0.05$) are denoted with different letters (i.e., a–g).

3.5. Total Polyphenol Content and Antiradical Activity of the Recovered Polyphenols

Extracting bioactive compounds from a sample is advantageous as it preserves their distinctive characteristics. It was necessary to determine whether the antioxidant properties of the extracted polyphenols were retained or altered by the CPE process. The selection of a method for quantifying total polyphenols, instead of total flavonoids, was chosen due to the fact that peach primarily consists not only of flavonoids (flavan-3-ols and anthocyanins), but also other phenolic acids (mostly chlorogenic acid) [51]. The Folin–Ciocalteu method is often subject to certain limitations, particularly in relation to potential interference from sugars and proteins. This issue is particularly relevant in the case of sugar-rich food products, such as honey [52]. Interference has been observed in different food products, including fruit juice [53] and vegetables [54]. Nevertheless, the impact of sugar and vitamin C levels on the majority of food items is typically insignificant [55]. So, the total polyphenol content (TPC) was quantified using the Folin–Ciocalteu method, with the results expressed as milligrams of gallic acid equivalents per liter (mg GAE/L). Meanwhile, the assessment of antiradical activity was performed using the DPPH$^{\bullet}$ test. According to the results, it was found that Tween 80 was the most efficient and cost-effective surfactant when used in a two-step CPE at 5% w/v. Consequently, it would be a more viable way for the peach industry where the surfactant would be used in bulk quantities. This is the reason why this specific surfactant was used instead of the natural surfactant lecithin, despite the abovementioned important advantages that lecithin provides. The outcomes of the two-step CPE are shown in Table 1. Statistically significant variations ($p < 0.05$) were observed only in the measurements related to the PW streams, but not in the measurements before and after the CPE procedures. Overall, both the initial sample and LPWS exhibited the highest TPC and A_{AR}.

Peels are also known to contain higher quantities of polyphenols, carotenoids, and total ascorbic acid than the flesh of peaches, plums, and nectarines [56]. The phytochemical composition found in the peel is typically two–three times greater than that found in the flesh, possibly due to the protective role polyphenols play against ultraviolet radiation, pathogens, and fruit predators [57]. Since peels are located on the exterior of the fruit, they are more prone to polyphenol formation [58].

High recovery yields were achieved using the CPE approach. The initial TPC in LPWS was measured at 603.2 mg GAE/L and the extraction yielded a 98.7% recovery. In TWS, the

initial TPC was measured at 50.3 mg GAE/L and the extraction yielded 98.2% recovery. It is important to highlight an observed pattern in both PW streams. Cloud point extraction was more efficient in the TWS than in the LPWS. This difference may be attributed to the statistically significant ($p < 0.05$) higher polyphenol content in the LPWS, making the recovery process more demanding.

Table 1. Total polyphenol content (TPC) and antiradical activity (A_{AR}) expressed as % DPPH$^{\bullet}$ scavenging in LPWS and TWS, before and after the CPE procedure (with Tween 80).

Sample Waste	Phase	TPC (mg GAE/L)	% DPPH$^{\bullet}$ Scavenging
LPWS	Initial	603.2 ± 21.1^{a}	65.4 ± 4.7^{a}
	CPE extract	595.7 ± 20.5^{a}	63.9 ± 1.9^{a}
TWS	Initial	50.3 ± 1.3^{b}	6.1 ± 0.2^{b}
	CPE extract	49.4 ± 1^{b}	5.7 ± 0.3^{b}

The data present mean values ± standard deviation of three replicates. Statistically significant differences ($p < 0.05$) between the samples are indicated by different superscript letters (e.g., $^{a,\ b}$).

Our results could be compared with Redondo et al. [59] who studied the differences in antioxidant capacity and phenolic content between thin and ripe peaches (*cv. UFO-3*). They measured approximately 500–900 mg GAE/kg of fresh weight, with riper fruits having a higher polyphenol value. The TWS sample initially recorded an average of 50.3 mg GAE/L. Concerning antiradical activity measurements, LPWS recorded 65.4% $A_{AR,}$ and the sample after the CPE procedure recorded a 2.29% decrease. In TWS, the sample initially recorded 6.1% A_{AR} and 5.7% after CPE. The results suggest that the recovery of polyphenols from industrial PW is valuable, particularly the LPWS since they exhibit increased rates of DPPH$^{\bullet}$ scavenging activity. Our findings align with the study conducted by Manzoor et al. [60] who examined the variability in minerals, polyphenols, and antioxidant activity between the peel and pulp of various peach varieties (Shireen, Golden, and Shahpasand). The A_{AR} of 80% methanolic extracts of peels ranged from 66.8–76.5%. It is important to point out that from an industrial point of view, the utilization of the LPWS would be a more cost-effective and practical solution than the TWS. The first stream showed a statistically significant difference ($p < 0.05$) in TPC and A_{AR}, up to 10 times greater in each case. In addition, the substantially large volume of TWS makes it difficult to handle and requires a correspondingly larger amount of surfactant.

To highlight the financial and operational importance of the CPE method in the peach industry, it would be interesting to compare it with other methods for the recovery of polyphenols from peach by-products. In our case, ~595 mg of polyphenols were recovered from 1 L of LTWS, with an overall percentage recovery of ~98%. Theoretically, the process would require 5% w/v of Tween 80 in a two-step CPE process (10% w/v in total), thus 100 g surfactant per liter of waste. A study conducted by Stramarkou et al. [61] examined an enhanced solid-liquid extraction with different combinations of extraction time, ultrasonic power, and solvent-to-dry solid ratio in order to determine the optimal extraction conditions. Based on the results of the study, it was determined that a natural deep eutectic solvent (choline chloride with lactic acid diluted with water 7:3) exhibited the highest efficacy as a solvent for the extraction of peach pulp waste. This was evidenced by an extraction yield of 17.13% and ~16 mg GAE/g of dry solid. In a theoretical conversion of this value to mg/L, it would equal to ~16,000 mg GAE/L. This major difference in comparison to our sample is anticipated since in the above study the sample was dried, so the amount of polyphenols was concentrated in a dried mass. The extraction yield might be low, but the polyphenols obtained are considerably higher than in our study. However, this process could require laborious methods such as drying and the use of ultrasonic and microwave-assisted extraction, which on an industrial scale is more difficult to achieve. Furthermore, in the above study the liquid-to-solid ratio was 10 mL/g. In a peach industry producing tons of waste, it would require a huge amount of extraction solvent, which would result in high cost. Furthermore, other methodologies such as aqueous two-phase extraction are

predominantly restricted to laboratory scale and have not yet been widely implemented on an industrial scale [12,62].

4. Conclusions

In this study, CPE was tested to recover polyphenols from PW. Four food-grade surfactants (Genapol X-080, PEG 8000, Tween 80, and lecithin) were tested at varied concentrations ranging from 2–10% w/v. To assess the effectiveness of this method, two PW streams (i.e., LPWS and TWS) were investigated. The recovery of polyphenols was found to be proportional to the surfactant concentration and extraction steps in both PW streams. Among the surfactants tested, Tween 80 exhibited the highest efficiency, achieving satisfactory recovery yields in both PW streams. LPWS was found to have significantly higher TPC and A_{AR}. Therefore, LPWS holds greater potential for valorization due to its higher polyphenol content and smaller waste volume, making it more manageable. The utilization of CPE with Tween 80 as a low-toxicity surfactant emerges as a rapid, straightforward, and inexpensive method for the recovery of polyphenols from PW. These recovered polyphenols could be directly utilized as natural antioxidants in food products. These findings have the potential to impact the production of natural and sustainable food additives, promoting the use of valuable by-products from the peach industry.

Author Contributions: Conceptualization, I.G. and S.I.L.; methodology, V.A. and T.C.; software, V.A.; validation, I.G. and V.A.; formal analysis, I.G., V.A., E.B. and T.C.; investigation, I.G. and V.A.; resources, S.I.L.; data curation, I.G. and V.A.; writing—original draft preparation, I.G., V.A., T.C. and D.K.; writing—review and editing, I.G., V.A., T.C., D.K., E.B., O.G., G.D.N. and S.I.L.; visualization, V.A.; supervision, O.G., G.D.N. and S.I.L.; project administration, S.I.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank ELBAK S.A. fruit canning factory, Falani, Larissa, Greece, for donating peach wastewater and peels.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Russ, W.; Meyer-Pittroff, R. Utilizing Waste Products from the Food Production and Processing Industries. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 57–62. [CrossRef]
2. Breida, M.; Younssi, S.A.; Ouammou, M.; Bouhria, M.; Hafsi, M.; Breida, M.; Younssi, S.A.; Ouammou, M.; Bouhria, M.; Hafsi, M. Pollution of Water Sources from Agricultural and Industrial Effluents: Special Attention to NO_3^-, Cr(VI), and Cu(II). In *Water Chemistry*; IntechOpen: London, UK, 2019; ISBN 978-1-78985-558-6.
3. Sathya, K.; Nagarajan, K.; Carlin Geor Malar, G.; Rajalakshmi, S.; Raja Lakshmi, P. A Comprehensive Review on Comparison among Effluent Treatment Methods and Modern Methods of Treatment of Industrial Wastewater Effluent from Different Sources. *Appl. Water Sci.* **2022**, *12*, 70. [CrossRef]
4. Barbera, M.; Gurnari, G. *Wastewater Treatment and Reuse in the Food Industry*; Springer: Berlin/Heidelberg, Germany, 2018; ISBN 3-319-68442-6.
5. Federici, F.; Fava, F.; Kalogerakis, N.; Mantzavinos, D. Valorisation of Agro-industrial By-products, Effluents and Waste: Concept, Opportunities and the Case of Olive Mill Wastewaters. *J. Chem. Technol. Biotechnol. Int. Res. Process Environ. Clean Technol.* **2009**, *84*, 895–900. [CrossRef]
6. De Leonardis, A.; Macciola, V.; Lembo, G.; Aretini, A.; Nag, A. Studies on Oxidative Stabilisation of Lard by Natural Antioxidants Recovered from Olive-Oil Mill Wastewater. *Food Chem.* **2007**, *100*, 998–1004. [CrossRef]
7. Malik, S.N.; Ghosh, P.C.; Vaidya, A.N.; Waindeskar, V.; Das, S.; Mudliar, S.N. Comparison of Coagulation, Ozone and Ferrate Treatment Processes for Color, COD and Toxicity Removal from Complex Textile Wastewater. *Water Sci. Technol. J. Int. Assoc. Water Pollut. Res.* **2017**, *76*, 1001–1010. [CrossRef]
8. Park, C.; Hong, S.-W.; Chung, T.H.; Choi, Y.-S. Performance Evaluation of Pretreatment Processes in Integrated Membrane System for Wastewater Reuse. *Desalination* **2010**, *250*, 673–676. [CrossRef]

9. Adebayo, F.O.; Obiekezie, S.O. Microorganisms in Waste Management. *Res. J. Sci. Technol.* **2018**, *10*, 28. [CrossRef]
10. Peng, X.; Xu, H.; Yuan, X.; Leng, L.; Meng, Y. Mixed Reverse Micellar Extraction and Effect of Surfactant Chain Length on Extraction Efficiency. *Sep. Purif. Technol.* **2016**, *160*, 117–122. [CrossRef]
11. Zhang, W.; Liu, X.; Fan, H.; Zhu, D.; Wu, X.; Huang, X.; Tang, J. Separation and Purification of Alkaloids from Sophora Flavescens Ait. by Focused Microwave-Assisted Aqueous Two-Phase Extraction Coupled with Reversed Micellar Extraction. *Ind. Crops Prod.* **2016**, *86*, 231–238. [CrossRef]
12. Pereira, J.F.B.; Freire, M.G.; Coutinho, J.A.P. Aqueous Two-Phase Systems: Towards Novel and More Disruptive Applications. *Fluid Phase Equilibria* **2020**, *505*, 112341. [CrossRef]
13. Xie, M.; Shon, H.K.; Gray, S.R.; Elimelech, M. Membrane-Based Processes for Wastewater Nutrient Recovery: Technology, Challenges, and Future Direction. *Water Res.* **2016**, *89*, 210–221. [CrossRef]
14. Chen, Y.; Du, K.; Li, J.; Bai, Y.; An, M.; Tan, Z.; Chang, Y. A Green and Efficient Method for the Preconcentration and Determination of Gallic Acid, Bergenin, Quercitrin, and Embelin from *Ardisia Japonica* Using Nononic Surfactant Genapol X-080 as the Extraction Solvent. *Int. J. Anal. Chem.* **2018**, *2018*, e1707853. [CrossRef] [PubMed]
15. Al_Saadi, M.R.; Al-Garawi, Z.S.; Thani, M.Z. Promising Technique, Cloud Point Extraction: Technology & Applications. *J. Phys. Conf. Ser.* **2021**, *1853*, 012064. [CrossRef]
16. Arya, S.S.; Kaimal, A.M.; Chib, M.; Sonawane, S.K.; Show, P.L. Novel, Energy Efficient and Green Cloud Point Extraction: Technology and Applications in Food Processing. *J. Food Sci. Technol.* **2019**, *56*, 524–534. [CrossRef] [PubMed]
17. Racheva, R.; Rahlf, A.F.; Wenzel, D.; Müller, C.; Kerner, M.; Luinstra, G.A.; Smirnova, I. Aqueous Food-Grade and Cosmetic-Grade Surfactant Systems for the Continuous Countercurrent Cloud Point Extraction. *Sep. Purif. Technol.* **2018**, *202*, 76–85. [CrossRef]
18. Yazdi, A.S. Surfactant-Based Extraction Methods. *TrAC Trends Anal. Chem.* **2011**, *30*, 918–929. [CrossRef]
19. Sharma, S.; Kori, S.; Parmar, A. Surfactant Mediated Extraction of Total Phenolic Contents (TPC) and Antioxidants from Fruits Juices. *Food Chem.* **2015**, *185*, 284–288. [CrossRef]
20. Aires, A.; Carvalho, R.; Saavedra, M.J. Reuse Potential of Vegetable Wastes (Broccoli, Green Bean and Tomato) for the Recovery of Antioxidant Phenolic Acids and Flavonoids. *Int. J. Food Sci. Technol.* **2017**, *52*, 98–107. [CrossRef]
21. Albuquerque, B.R.; Heleno, S.A.; Oliveira, M.B.P.P.; Barros, L.; Ferreira, I.C.F.R. Phenolic Compounds: Current Industrial Applications, Limitations and Future Challenges. *Food Funct.* **2021**, *12*, 14–29. [CrossRef]
22. Kant, R. A Review on Peach (*Prunus persica*): An Asset of Medicinal Phytochemicals. *Int. J. Res. Appl. Sci. Eng. Technol.* **2018**, *6*, 2186–2200. [CrossRef]
23. Alvarez-Parrilla, E.; De La Rosa, L.A.; González-Aguilar, G.A.; Ayala-Zavala, J.F. Phytochemical Composition and Health Aspects of Peach Products. In *Dried Fruits*; Alasalvar, C., Shahidi, F., Eds.; Blackwell Publishing Ltd.: Oxford, UK, 2013; pp. 309–324. ISBN 978-1-118-46466-3.
24. Chang, S.; Tan, C.; Frankel, E.N.; Barrett, D.M. Low-Density Lipoprotein Antioxidant Activity of Phenolic Compounds and Polyphenol Oxidase Activity in Selected Clingstone Peach Cultivars. *J. Agric. Food Chem.* **2000**, *48*, 147–151. [CrossRef] [PubMed]
25. Dimou, C.; Karantonis, H.C.; Skalkos, D.; Koutelidakis, A.E. Valorization of Fruits By-Products to Unconventional Sources of Additives, Oil, Biomolecules and Innovative Functional Foods. *Curr. Pharm. Biotechnol.* **2019**, *20*, 776–786. [CrossRef] [PubMed]
26. Monagas, M.; Garrido, I.; Lebrón-Aguilar, R.; Bartolome, B.; Gómez-Cordovés, C. Almond (*Prunus Dulcis* (Mill.) D.A. Webb) Skins as a Potential Source of Bioactive Polyphenols. *J. Agric. Food Chem.* **2007**, *55*, 8498–8507. [CrossRef] [PubMed]
27. Colak Gunes, N.; Gungor, E.; Gunes, M. Solar Process Heat for Sustainable Production of Concentrated Peach Puree. *J. Food Process Eng.* **2021**, *44*, e13744. [CrossRef]
28. Santana, C.M.; Ferrera, Z.S.; Rodríguez, J.J.S. Use of Non-Ionic Surfactant Solutions for the Extraction and Preconcentration of Phenolic Compounds in Water Prior to Their HPLC-UV Detection. *Analyst* **2002**, *127*, 1031–1037. [CrossRef]
29. Sosa Ferrera, Z.; Padrón Sanz, C.; Mahugo Santana, C.; Santana Rodríguez, J.J. The Use of Micellar Systems in the Extraction and Pre-Concentration of Organic Pollutants in Environmental Samples. *TrAC Trends Anal. Chem.* **2004**, *23*, 469–479. [CrossRef]
30. Chatzilazarou, A.; Katsoyannos, E.; Gortzi, O.; Lalas, S.; Paraskevopoulos, Y.; Dourtoglou, E.; Tsaknis, J. Removal of Polyphenols from Wine Sludge Using Cloud Point Extraction. *J. Air Waste Manag. Assoc.* **2010**, *60*, 454–459. [CrossRef]
31. Chatzilazarou, A.; Katsoyannos, E.; Lagopoulou, M.; Tsaknis, J. Application of Cloud Point Extraction with the Aid of Genapol X-080 in the Pre-Concentration of Lycopene and Total Carotenoids from Red Fleshed Orange. *Nutrition* **2011**, *35*, 10.
32. Katsoyannos, E.; Chatzilazarou, A.; Gortzi, O.; Lalas, S.; Konteles, S.; Tataridis, P. Application of Cloud Point Extraction Using Surfactants in the Isolations of Physical Antioxidants (Phenols) from Olive Mill Wastewater. *Fresenius Environ. Bull.* **2006**, *15*, 4.
33. Tsaknis, J.; Lalas, S. Extraction and Identification of Natural Antioxidant from Sideritis Euboea (Mountain Tea). *J. Agric. Food Chem.* **2005**, *53*, 6375–6381. [CrossRef]
34. Kiai, H.; Raiti, J.; El-Abbassi, A.; Hafidi, A. Recovery of Phenolic Compounds from Table Olive Processing Wastewaters Using Cloud Point Extraction Method. *J. Environ. Chem. Eng.* **2018**, *6*, 1569–1575. [CrossRef]
35. El-Abbassi, A.; Kiai, H.; Raiti, J.; Hafidi, A. Cloud Point Extraction of Phenolic Compounds from Pretreated Olive Mill Wastewater. *J. Environ. Chem. Eng.* **2014**, *2*, 1480–1486. [CrossRef]
36. Gortzi, O.; Lalas, S.; Chatzilazarou, A.; Katsoyannos, E.; Papaconstandinou, S.; Dourtoglou, E. Recovery of Natural Antioxidants from Olive Mill Wastewater Using Genapol-X080. *J. Am. Oil Chem. Soc.* **2008**, *85*, 133–140. [CrossRef]

37. Paleologos, E.K.; Giokas, D.L.; Karayannis, M.I. Micelle-Mediated Separation and Cloud-Point Extraction. *TrAC Trends Anal. Chem.* **2005**, *24*, 426–436. [CrossRef]
38. Kori, S. Cloud Point Extraction Coupled with Back Extraction: A Green Methodology in Analytical Chemistry. *Forensic Sci. Res.* **2021**, *6*, 19–33. [CrossRef]
39. He, J.; Zhao, Z.; Shi, Z.; Zhao, M.; Li, Y.; Chang, W. Analysis of Isoflavone Daidzein in Puerariae Radix with Micelle-Mediated Extraction and Preconcentration. *J. Agric. Food Chem.* **2005**, *53*, 518–523. [CrossRef]
40. Ibrahim, M.; Ramadan, E.; Elsadek, N.E.; Emam, S.E.; Shimizu, T.; Ando, H.; Ishima, Y.; Elgarhy, O.H.; Sarhan, H.A.; Hussein, A.K.; et al. Polyethylene Glycol (PEG): The Nature, Immunogenicity, and Role in the Hypersensitivity of PEGylated Products. *J. Control. Release* **2022**, *351*, 215–230. [CrossRef]
41. Giovanoudis, I.; Athanasiadis, V.; Chatzimitakos, T.; Kalompatsios, D.; Bozinou, E.; Gortzi, O.; Nanos, G.D.; Lalas, S.I. Implementation of Cloud Point Extraction Using Surfactants in the Recovery of Polyphenols from Apricot Cannery Waste. *Eng* **2023**, *4*, 1225–1235. [CrossRef]
42. Kaur, G.; Mehta, S.K. Developments of Polysorbate (Tween) Based Microemulsions: Preclinical Drug Delivery, Toxicity and Antimicrobial Applications. *Int. J. Pharm.* **2017**, *529*, 134–160. [CrossRef] [PubMed]
43. World Health Organization; Food and Agriculture Organization of the United Nations; Joint FAO/WHO Expert Committee on Food Additives. Meeting (80th: 2015, R., Italy). In *Evaluation of Certain Food Additives and Contaminants: Eightieth Report of the Joint FAO/WHO Expert Committee on Food Additives*; WHO Technical Report Series 995; World Health Organization: Geneva, Switzerland, 2016; ISBN 978-92-4-120995-3.
44. Katsoyannos, E.; Gortzi, O.; Chatzilazarou, A.; Athanasiadis, V.; Tsaknis, J.; Lalas, S. Evaluation of the Suitability of Low Hazard Surfactants for the Separation of Phenols and Carotenoids from Red-Flesh Orange Juice and Olive Mill Wastewater Using Cloud Point Extraction. *J. Sep. Sci.* **2012**, *35*, 2665–2670. [CrossRef]
45. Stamatopoulos, K.; Katsoyannos, E.; Chatzilazarou, A. Antioxidant Activity and Thermal Stability of Oleuropein and Related Phenolic Compounds of Olive Leaf Extract after Separation and Concentration by Salting-Out-Assisted Cloud Point Extraction. *Antioxidants* **2014**, *3*, 229–244. [CrossRef]
46. Wang, M.; Yan, W.; Zhou, Y.; Fan, L.; Liu, Y.; Li, J. Progress in the Application of Lecithins in Water-in-Oil Emulsions. *Trends Food Sci. Technol.* **2021**, *118*, 388–398. [CrossRef]
47. European Parliament, Council of the European Union. *Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on Food Additives (Text with EEA Relevance)*; European Parliament, Council of the European Union: Brussels, Belgium, 2008.
48. van Nieuwenhuyzen, W. Lecithin and Other Phospholipids. In *Surfactants from Renewable Resources*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2010; pp. 191–212. ISBN 978-0-470-68660-7.
49. Alibade, A.; Batra, G.; Bozinou, E.; Salakidou, C.; Lalas, S. Optimization of the Extraction of Antioxidants from Winery Wastes Using Cloud Point Extraction and a Surfactant of Natural Origin (Lecithin). *Chem. Pap.* **2020**, *74*, 4517–4524. [CrossRef]
50. Karadag, A.; Kayacan Cakmakoglu, S.; Metin Yildirim, R.; Karasu, S.; Avci, E.; Ozer, H.; Sagdic, O. Enrichment of Lecithin with Phenolics from Olive Mill Wastewater by Cloud Point Extraction and Its Application in Vegan Salad Dressing. *J. Food Process. Preserv.* **2022**, *46*, e16645. [CrossRef]
51. Zhao, X.; Zhang, W.; Yin, X.; Su, M.; Sun, C.; Li, X.; Chen, K. Phenolic Composition and Antioxidant Properties of Different Peach [*Prunus persica* (L.) Batsch] Cultivars in China. *Int. J. Mol. Sci.* **2015**, *16*, 5762–5778. [CrossRef]
52. Lawag, I.L.; Nolden, E.S.; Schaper, A.A.M.; Lim, L.Y.; Locher, C. A Modified Folin-Ciocalteu Assay for the Determination of Total Phenolics Content in Honey. *Appl. Sci.* **2023**, *13*, 2135. [CrossRef]
53. Ma, S.; Kim, C.; Neilson, A.P.; Griffin, L.E.; Peck, G.M.; O'Keefe, S.F.; Stewart, A.C. Comparison of Common Analytical Methods for the Quantification of Total Polyphenols and Flavanols in Fruit Juices and Ciders. *J. Food Sci.* **2019**, *84*, 2147–2158. [CrossRef]
54. Muñoz-Bernal, Ó.A.; Torres-Aguirre, G.A.; Núñez-Gastélum, J.A.; de la Rosa, L.A.; Rodrigo-García, J.; Ayala-Zavala, J.F.; Álvarez-Parrilla, E. New approach to the interaction of Folin-Ciocalteu reagent with sugars during total polyphenol quantification. *TIP Rev. Espec. Cienc. Quím.-Biológicas* **2017**, *20*, 23–28. [CrossRef]
55. Sánchez-Rangel, J.C.; Benavides, J.; Heredia, J.B.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. The Folin–Ciocalteu Assay Revisited: Improvement of Its Specificity for Total Phenolic Content Determination. *Anal. Methods* **2013**, *5*, 5990–5999. [CrossRef]
56. Tomás-Barberán, F.A.; Gil, M.I.; Cremin, P.; Waterhouse, A.L.; Hess-Pierce, B.; Kader, A.A. HPLC−DAD−ESIMS Analysis of Phenolic Compounds in Nectarines, Peaches, and Plums. *J. Agric. Food Chem.* **2001**, *49*, 4748–4760. [CrossRef]
57. Ignat, I.; Volf, I.; Popa, V.I. A Critical Review of Methods for Characterisation of Polyphenolic Compounds in Fruits and Vegetables. *Food Chem.* **2011**, *126*, 1821–1835. [CrossRef] [PubMed]
58. Barros, H.R.D.M.; Ferreira, T.A.P.D.C.; Genovese, M.I. Antioxidant Capacity and Mineral Content of Pulp and Peel from Commercial Cultivars of Citrus from Brazil. *Food Chem.* **2012**, *134*, 1892–1898. [CrossRef]
59. Redondo, D.; Gimeno, D.; Calvo, H.; Venturini, M.E.; Oria, R.; Arias, E. Antioxidant Activity and Phenol Content in Different Tissues of Stone Fruits at Thinning and at Commercial Maturity Stages. *Waste Biomass Valorization* **2021**, *12*, 1861–1875. [CrossRef]
60. Manzoor, M.; Anwar, F.; Mahmood, Z.; Rashid, U.; Ashraf, M. Variation in Minerals, Phenolics and Antioxidant Activity of Peel and Pulp of Different Varieties of Peach (*Prunus persica* L.) Fruit from Pakistan. *Molecules* **2012**, *17*, 6491–6506. [CrossRef]

61. Stramarkou, M.; Oikonomopoulou, V.; Panagiotopoulou, M.; Papadaki, S.; Krokida, M. Sustainable Valorisation of Peach and Apricot Waste Using Green Extraction Technique with Conventional and Deep Eutectic Solvents. *Resources* **2023**, *12*, 72. [CrossRef]
62. Grilo, A.L.; Raquel Aires-Barros, M.; Azevedo, A.M. Partitioning in Aqueous Two-Phase Systems: Fundamentals, Applications and Trends. *Sep. Purif. Rev.* **2016**, *45*, 68–80. [CrossRef]