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Sustainable Exploitation of Waste Orange Peels: Enrichment of Commercial Seed Oils and the Effect on Their Oxidative Stability

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Abstract: The current project aimed at examining the effect of the enrichment of commercial seed oils with waste orange peel (WOP) extracts on their polyphenolic profiles and resistance against oxidation. Polyphenol-containing WOP extracts were produced using a novel combination of ethanol and triacetin, and they were incorporated into seed oils (sunflower, soybean, corn oil), at a level of 36.87 mg per kg of oil. The oils were then stored at 60 °C, for 58 days. By performing a Rancimat test, it was shown that enrichment of sunflower, soybean, and corn oils with WOP extracts did not provoke any prooxidant effects, but, to the contrary, exerted an antioxidant action, with protection factors varying from 1.01 to 1.61. Furthermore, in all cases examined, it was demonstrated that, during the storage period, the stabilizing effect of WOP extract against oxidation was comparable to that observed in oil samples containing 200 mg BHT per kg oil. This outcome was ascertained by measuring the onset of peroxide value, thiobarbituric-acid-reactive substances, and the TOTOX value. Furthermore, it was revealed that the Trolox-equivalent antiradical activity of the enriched oils exhibited a decline at the end of the examination period, a fact most probably attributed to the depletion of the antioxidants occurring in the oils. It was concluded that the method proposed might be a means of stabilizing commercial seed oils against oxidation, and of enhancing their nutritional value by enriching them with natural polyphenols.

Keywords: antioxidants; food waste valorization; oil oxidation; orange peels; polyphenols

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Citation: Kalompatsios, D.; Athanasiadis, V.; Chatzimitakos, T.; Palaiogiannis, D.; Lalas, S.I.; Makris, D.P. Sustainable Exploitation of Waste Orange Peels: Enrichment of Commercial Seed Oils and the Effect on Their Oxidative Stability. *Waste* **2023**, *1*, 761–774. https://doi.org/10.3390/waste1030045

Academic Editors: Catherine N. Mulligan and Apostolos Giannis

Received: 22 June 2023
Revised: 31 July 2023
Accepted: 30 August 2023
Published: 2 September 2023

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1. Introduction

The rapid expansion of the world's population and the associated intensification of land cultivation and food production has led to eco-system degradation and environmental aggravation at unprecedented levels. It is now widely acknowledged that the linear economy models have failed to meet the requirements for resource conservation and balanced development; thus, bioeconomy and cyclic economy strategies are gaining significant acceptance, as the only routes leading to sustainability, bioresource efficiency, and higher profitability for the agri-food sector. Within such a frame, biorefinery approaches constitute the pillars of waste recycling and reuse policies, and effective biomass valorization [1,2].

The side streams originating from food processing may be considered as resources of exceptional richness in bioactive phytochemicals of various structures and forms, such as polysaccharides, essential oils, pigments, antioxidants, etc. Polyphenols are a group of secondary plant metabolites exhibiting a vast spectrum of versatile bioactivities, and they are considered some of the most important, high-valued-added substances that can be recovered from industrial food processing residues [3]. Polyphenols occur in a multitude of structures, and encompass biological properties associated with antioxidant, anti-inflammatory, antimicrobial, cardioprotective, and chemoprotective activities [4]. Thus, the exploitation of polyphenol-rich biomass in a wide range of industrial applications, mainly

in the food, pharmaceutical, and cosmetics sectors, has become a primary focus for the relevant industries [5,6].

Citrus crops are the largest fruit crops in the world, and orange production accounts for 60% of the total citrus amount. Oranges are primarily processed into juice, with a yield of about 50% on a fresh fruit weight basis. The remaining 50% consists of waste peel, seeds, pulp, and discarded defected orange fruits [7]. Orange world production was 46 million metric tons in 2019, of which approximately 37% were further processed into various commodities. These data point emphatically to orange processing side streams as a major pool of food processing waste. Thus, strategies embracing reuse/valorization of orange processing residues targeting at effective polyphenol recovery become imperative, considering that numerous studies have well documented the bioactivities of citrus flavonoids, which are currently used as nutraceuticals and health supplements [8].

Another option of orange peel polyphenol valorization would be their use as efficient food antioxidants, as previously reported for various oils and fat-containing products [9–11]. Orange peels might be a suitable candidate for such a task, given their flavonoid composition, consisting of significant amounts of polymethylated (lipophilic) flavonoids [12]. These compounds could be easily incorporated into oils, and act as stabilizers against oxidative deterioration. However, investigations in this field are extremely limited, and therefore, the feasibility of such a use for waste orange peel (WOP) is largely unexplored. This being the conceptual basis, the current examination aimed at studying WOP as a raw material to retrieve polyphenols, which then could be added to commercial seed oils as natural antioxidants. To this end, a novel approach was used for WOP polyphenol extraction, employing mixtures of triacetin and ethanol. After ethanol removal, the remaining polyphenol-enriched, triacetin solution was directly added into seed oils, since triacetin is a very short-chain fatty acid, triacylglycerol, and it is completely miscible with oils. Then, the oils were tested for stability by performing various tests. To the best of the authors' knowledge, this study is the first report on such an approach for commercial oil fortification with natural antioxidants.

2. Materials and methods

2.1. Reagents and Chemicals

Ammonium iron (II) sulfate, thiobarbituric acid (TBA), trichloroacetic acid, hydrochloric acid (37%), and glacial acetic acid were purchased from Panreac (Barcelona, Spain). Triacetin was from Glentham Life Sciences Ltd. (Corsham, UK). Gallic acid monohydrate, absolute ethanol, and Folin–Ciocalteu reagent were from Panreac (Barcelona, Spain). Neochlorogenic acid, chlorogenic acid, caffeic acid, luteolin 7-*O*-rutinoside, ferulic acid, narirutin, hesperidin, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), methanol, hexane, and cyclohexane were obtained from Sigma-Aldrich (St. Louis, MA, USA). Dichloromethane, ethyl acetate, and isooctane were obtained from Carlo Erba (Vaul de Reuil, France). Sodium acetate anhydrous, ammonium thiocyanate and sodium carbonate anhydrous were from Penta (Prague, Czechia). Hydrogen peroxide (35%) was obtained from Chemco (Malsch, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Glentham Life Sciences (Corsham, UK). Solvents used for chromatographic determinations were HPLC grade.

2.2. Seed Oil Procurement

Sunflower oil, soybean oil, and corn oil were purchased from a local grocery store (Karditsa, Greece). The oils were stored in the dark, at 23 °C, and they were used immediately after opening.

2.3. Collection and Handling of Waste Orange Peels (WOP)

To obtain a realistic WOP sample that would be a representative food-processing residue, orange fruit residues consisting essentially of orange peels were collected from various catering facilities (Larissa, Greece) that regularly process orange fruits on a daily

basis to produce fresh-squeezed orange juice. After collection, which was accomplished within 3 consecutive days, transport to the laboratory was carried out within 2 h. Upon receipt, the material was screened to remove apparently infected peels and foreign matter, and then dried in a laboratory oven (Binder BD56, Bohemia, NY, USA). Conditions of drying, as well as further handling and storage, have been previously described in detail [13].

2.4. Extraction Procedure and Protocol

All extractions were performed using a constant liquid-to-solid ratio of 20 mL g^{-1}, and a stirring speed of 500 rpm. The solvent (20 mL), composed of various ratios of ethanol/triacetin, was introduced in a 25 mL Duran™ bottle and heated at 70 °C, for 180 min, by means of an oil bath, placed on a temperature-controlled hotplate (Witeg, Wertheim, Germany). After the elapse of 10 min to ensure that the solvent acquired the predetermined temperature, the pulverized WOP (1 g) was added. After each extraction, the mixtures were centrifuged at 10,000× *g* to separate cell debris and obtain a clear supernatant.

2.5. Seed Oil Enrichment and Treatment

An amount of 5 g of WOP was extracted with 50 mL ethanol/triacetin mixture of optimum composition, as described in paragraph 2.4. Then, ethanol was removed *in vacuo*, at 40 °C. The remaining triacetin solution was centrifuged 5 min at 4500× *g* and left overnight at room temperature to observe any possible precipitate. Once extract stability was ascertained, 99 g of each seed oil was combined with 1 g triacetin solution, mixed well by hand shaking, and placed in a water bath, at 60 °C, in the dark. Sampling was accomplished every 3 days for a period of 58 days, to carry out analyses pertaining to oil stability.

2.6. Determination of Total Polyphenols

Polyphenol extraction from oils was performed according to a previously described protocol [14], and Folin–Ciocalteu determination was executed based on a published methodology [15]. An accurate amount of 1 g of oil was diluted with 2 mL hexane and extracted with 2 mL of 60% methanol/water mixture. The mixture was shaken vigorously with a vortex apparatus and centrifuged at 4500 rpm for 5 min. Then, 0.1 mL of the aqueous phase was transferred along with 0.1 mL of Folin–Ciocalteu reagent in a 1.5 mL Eppendorf tube, and the reaction was left to proceed for 2 min. Following this, 0.80 mL of 5% *w*/*v* sodium carbonate solution was added, and the mixture was heated in a thermostated water bath (Falc Instruments LBS2, Treviglio, Italy), at 40 °C for 20 min. The absorbance was recorded at 740 nm in a Shimadzu UV-1700 PharmaSpec spectrophotometer (Kyoto, Japan), and the determination of total polyphenol concentration was accomplished with a gallic acid calibration curve (10–100 mg L^{-1}). The oil content in total polyphenols (C_{TP}) was expressed as mg gallic acid equivalents (GAE) per kg of oil.

2.7. Rancimat

For the Rancimat test, a Rancimat 743 (Metrohm LTD, Herisau, Switzerland) device was used, interfaced by 873 Biodiesel Rancimat (version 1.00) software. The device was operated at 110 °C with an air flow rate of 15 L h^{-1}, and the mass of the oil sample used was 3 g. After recording the induction period (in h) for each oil sample tested, the protection factor (PF) was determined as previously proposed [16]:

$$\mathrm{PF} = \frac{\text{Induction period of sample}}{\text{Induction period of control}} \quad (1)$$

2.8. Determination of Peroxide Value (PV)

The IDF standard method, 74A:1991 [17] was used to determine the peroxide values of all oil samples with some modifications. Briefly, 0.05 g of oil sample was dissolved in a 2 mL Eppendorf tube with 2 mL dichloromethane/ethanol (3:2, *v*/*v*) and vortexed for 2–4 s. Oil sample extract (20 µL) was mixed with 1960 µL of solvent (DCM/EtOH), ammonium thiocyanate solution (10 µL, 4 M in water) was added, and the sample was vortexed again for 2–4 s. Then, 10 µL of ammonium iron(II) sulfate solution (25.5 mM in 10 M HCl) was added, and the sample was mixed on a vortex for another 2–4 s. After a 5 min incubation at room temperature, the absorbance of the sample was measured at 500 nm against a suitable blank. PV was calculated using a hydrogen peroxide (H_2O_2) calibration curve that was constructed using the above procedure with six different concentrations (50–500 µmoL L^{-1} in DCM/EtOH). Results were expressed as mmoL H_2O_2 per kg of oil, using the following equation:

$$\text{PV} = \frac{C_{H_2O_2} \times V}{w} \tag{2}$$

where $C_{H_2O_2}$ is the concentration of H_2O_2 (in µmoL L^{-1}), V is the volume of the extraction medium (in L), and w is the weight of the reaction oil sample (in g).

2.9. Determination of Thiobarbituric Acid Reactive Substances (TBARS)

The assay for TBARS determination carried out was according to a previously published protocol [18]. An accurate mass of 0.1 g of oil was transferred into a 15 mL Falcon tube and 5 mL of thiobarbituric acid (TBA) reagent was added. The TBA reagent was prepared by mixing 15 g of trichloroacetic acid (TCA), 0.375 g of TBA, and 1.76 mL of 12 M HCl into a 100 mL volumetric flask, made to a final volume of 100 mL with deionized water. The reactants were mixed thoroughly and incubated in a water bath at 95 °C for 20 min. After incubation, the samples were chilled on ice for 5 min, 200 µL of chloroform was added, vortexed for 5 s, and then centrifuged at 4500× *g* for 10 min. The absorbance of the transparent supernatant was measured at 532 nm. The thiobarbituric acid reactive substances (TBARS) value was determined as mmoL of malondialdehyde equivalents (mmoL MDAE) per kg of oil, using a malondialdehyde calibration curve (15–300 µmoL/L in deionized water), as shown below:

$$\text{TBARS} = \frac{C_{\text{MDA}} \times V}{w} \tag{3}$$

where C_{MDA} is the concentration of malondialdehyde (in µmoL L^{-1}), V is the volume of the extraction medium (in L), and w the weight of the oil sample (in g).

2.10. Determination of p-Anisidine Value

The *p*-anisidine value was determined according to the ES ISO 6885:2012 method [19]. An exact weight of 0.5 g of oil sample was placed into a 10 mL volumetric flask, and the flask was made to the volume with isooctane. A volume of 1 mL of this solution was then mixed with 0.2 mL of glacial acetic acid, shaken vigorously, and kept for 10 min in dark. Following this, absorbance (A_0) at 350 nm was measured, using isooctane as a blank. Likewise, 1 mL of the oil solution was mixed with 0.2 mL of *p*-anisidine (0.5% in glacial acetic acid), and the absorbance (A_1) was read at 350 nm. This procedure was also repeated by replacing the oil sample with pure isooctane, and the absorbance (A_2) at 350 nm was recorded. The *p*-anisidine value (*p*-AnV) was calculated using the expression:

$$p\text{-AnV} = \frac{100\ Q\ V}{m}\ 0.24\ [A_1 - A_2 - A_0] = 12\left(\frac{A_1 - A_2 - A_0}{m}\right) \tag{4}$$

where Q is the concentration of the oil in the solution tested (0.05 g mL^{-1}), V is the volume of oil solution in isooctane (10 mL), and m is the mass of the oil tested (g); A_0 represents the absorbance of the unreacted test solution, A_1 is the absorbance of the reacted solution,

and A_2 is the absorbance of the blank; 0.24 is the correction factor for the dilution of the test solution with 0.2 mL of the reagent solution in acetic acid or pure acetic acid.

2.11. Determination of Antioxidant Capacity (TEAC)

TEAC was estimated as reported elsewhere [14]. In short, 0.5 g of oil was dissolved in 5 mL of ethyl acetate. Then, 0.05 mL of oil sample was mixed with 0.95 mL DPPH$^{\bullet}$ solution (100 µM in ethyl acetate) and shaken vigorously for 10 s. The absorbance at 515 nm was obtained immediately after mixing ($A_{515(i)}$) and after exactly 30 min ($A_{515(f)}$). A_{AR} was computed according to the equation:

$$\text{Inhibition } (\%) = \left(\frac{A_{515(i)} - A_{515(f)}}{A_{515(i)}} \right) \times 100 \quad (5)$$

Results were expressed as Trolox equivalent antioxidant capacity (TEAC), using a calibration curve (50–500 µM), constructed by plotting % inhibition against Trolox concentration (in µM).

2.12. Chromatographic Determinations

The tentative identification of didymin, sinensetin, nobiletin, and dimethylnobiletin in WOP extracts was accomplished with liquid chromatography–mass spectrometry. Settings pertaining to the elution program and acquisition of mass spectra have been given in an earlier study [20]. Dereplication based on UV–Vis and mass spectral data has been reported in detail elsewhere [21]. Quantification of the aforementioned substances was accomplished using a luteolin 7-*O*-glucoside calibration curve (R^2 = 0.9980). The chromatography equipment and specific details for the quantification of caffeic acid, ferulic acid, neochlorogenic acid, chlorogenic acid, narirutin, and hesperidin were as was previously published [22].

2.13. Statistical Processing and Analyses

Waste orange peel extraction, as well as seed oil enrichment and treatment, were carried out at least twice. All analytical determinations were performed in triplicate. Distribution analyses and linear regressions were computed with SigmaPlot™ 12.5 (Systat Software Inc., San Jose, CA, USA).

3. Results and Discussion

3.1. Extract Preparation and Polyphenolic Profile

The distinction of the most efficacious proportion of ethanol/triacetin with regard to polyphenol extraction from WOP was accomplished by switching the ethanol/triacetin ratio of the solvent, from 0 to 100%, by a 20% step. This approach enabled a detailed monitoring of the effect of ethanol on the total polyphenol yield (Y_{TP}). The results of this test are depicted in Figure 1, and it can be seen that a solvent system comprised of ethanol/triacetin 8/2 (v/v) gave a significantly higher Y_{TP} ($p < 0.05$) compared to all other systems assayed. Thus, WOP extracts produced with ethanol/triacetin (8/2) were collected and ethanol was removed in vacuo to generate a triacetin solution. This solution was then subjected to chromatographic analysis, to profile its polyphenolic composition, and in the trace monitored at 320 nm, nine peaks could be tentatively identified (Figure 2).

The quantitative analysis revealed that the extract was dominated by hesperidin, followed by narirutin, nobiletin, and didymin (Table 1).

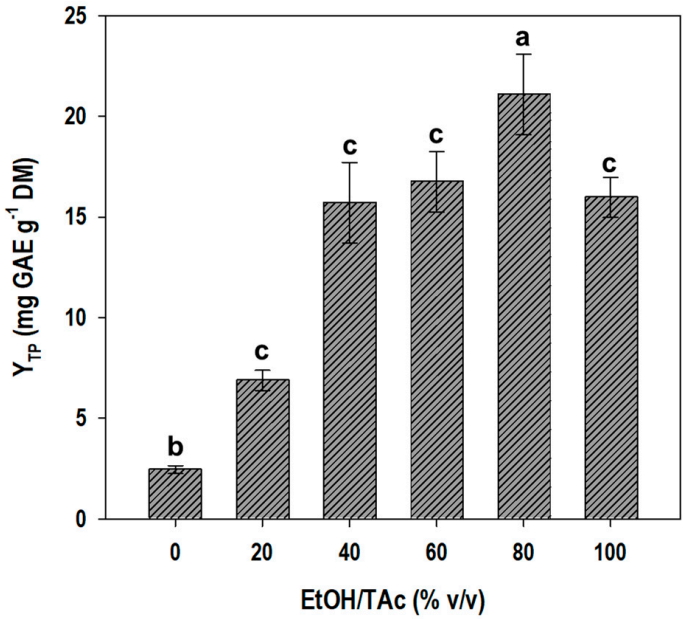


Figure 1. Bar diagram illustrating the yield in total polyphenols (Y_{TP}) as a function of ethanol (EtOH)/triacetin (TAc) volume ratio of the solvent systems tested. Bars designated with different letters (a, b, c) represent statistically different Y_{TP} values ($p < 0.05$). Error bars show the standard deviation.

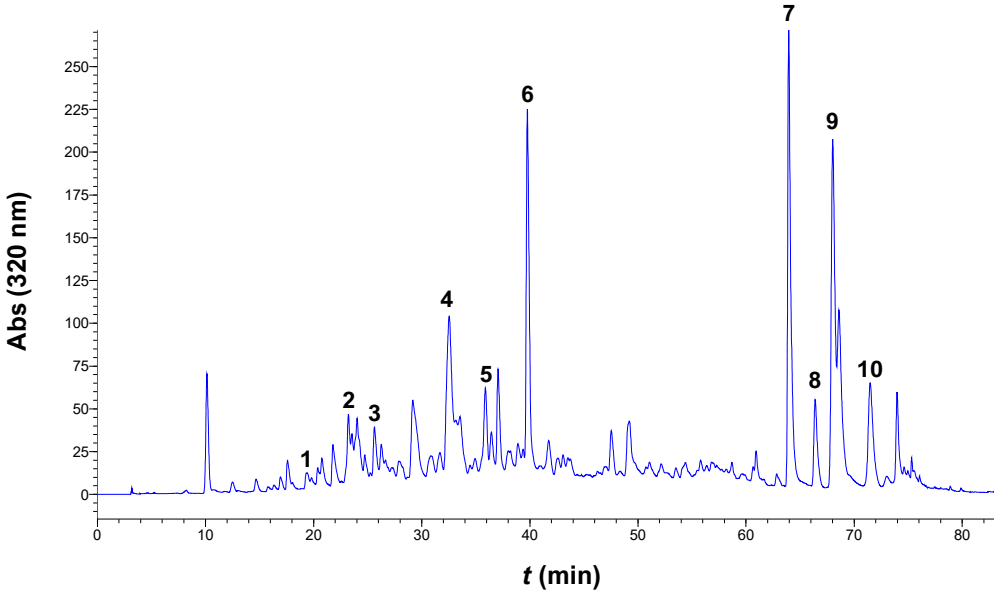


Figure 2. HPLC trace of a WOP triacetin extract, monitored at 320 nm. Peak assignment: 1, neochlorogenic acid; 2, chlorogenic acid; 3, caffeic acid; 4, ferulic acid; 5, narirutin; 6, hesperidin; 7, didymin; 8, sinensetin; 9, nobiletin; 10, dimethylnobiletin.

Table 1. Quantitative data on the content of major polyphenols occurring in the triacetin extract used for seed oil enrichment.

Polyphenol	Content (μg g^{-1} Extract) ± sd
Phenolic acids	
Neochlorogenic acid	16.34 ± 1.52
Chlorogenic acid	34.77 ± 2.22

Table 1. *Cont.*

Polyphenol	Content (μg g^{-1} Extract) ± sd
Caffeic acid	6.54 ± 0.58
Ferulic acid	105.47 ± 8.41
Total	*163.13*
Flavanones	
Narirutin	547.07 ± 41.23
Hesperidin	2037.64 ± 169.02
Total	2584.72 ± 187.73
Flavones	
Didymin	322.90 ± 28.00
Sinensetin	85.93 ± 7.89
Nobiletin	355.66 ± 30.18
Dimethylnobiletin	174.99 ± 12.33
Total	*939.48*
Sum	*3687.32*

3.2. Oil Enrichment and Protection Factor (PF)

Triacetin, being a very short fatty acid triacylglyerol, is completely miscible with oils, which are largely composed of triacylglycerol mixtures. On such a basis, the polyphenol-enriched triacetin extract was incorporated into commercially available sunflower oil (SnO), soybean oil (SbO), and corn oil (CnO), at a level of 1% (*w*/*w*). Based on the data of Table 1, this proportion would give a final total polyphenol content of 36.87 mg per kg of oil. As positive controls, oils were mixed with a triacetin solution of butylated hydroxytoluene (BHT) at the same level (1% *w*/*w*), to provide a final BHT content of 200 mg per kg of oil. Oils without any additive were also tested, to ascertain the effect of the WOP extract. The initial composition of the oils tested prior to enrichment, as well as indices pertaining to their oxidation status, are analytically presented in Table 2.

Table 2. Indices pertaining to oil composition and oxidative status prior to enrichment with WOP extract.

Index	Sample		
	SnO	SbO	CnO
C_{TP} (mg GAE kg^{-1} oil)	19.99 ± 5.66	12.58 ± 1.59	13.63 ± 3.14
PV (mm H_2O_2 kg^{-1} oil)	0.38 ± 0.08	1.77 ± 0.54	0.11 ± 0.04
TBARS (mmol MDE eq kg^{-1} oil)	0.52 ± 0.04	0.66 ± 0.04	0.71 ± 0.05
p-AnV	9.73 ± 1.02	2.34 ± 0.54	4.23 ± 0.89

To assess the protection that the addition of WOP extract could possibly exert on oil oxidation, enriched and control samples were subjected to a Rancimat test, to determine both the induction period and the PF (Table 3). For SnO, the addition of WOP extract prolonged the induction period, giving a PF of 1.17. The addition of BHT was more effective, providing a PF of 1.61. The pattern observed for both SbO and CnO was likewise, suggesting that for all three oils examined, the addition of WOP exerted a protective effect, although for CnO this effect was marginal, with a PF of 1.01. Previous investigations reported a PF of 1.36, upon enrichment of corn oil with *Citrus aurantium* peel extract, which afforded a total polyphenol content of 1.22 mg GAE kg^{-1} of oil [23]. PF values reported for SnO enriched with various olive leaf extracts varied from 0.16 to 2.08, whereas for SbO, the PF varied from 0.23 to 1.41 [24].

Table 3. Induction period and protection factor determined for the oil samples, on the basis of Rancimat test.

Sample	Induction Period (h)	PF
SnO	1.2	-
SnO + WOP	1.4	1.17
SnO + BHT	1.9	1.61
SbO	5.8	
SbO + WOP	6.2	1.07
SbO + BHT	6.8	1.17
CnO	7.3	
CnO + WOP	7.4	1.01
CnO + BHT	8.0	1.09

Other investigations on the enrichment of SnO with olive leaf extract reported a PF of 1.54 [25]. Furthermore, a PF of 2.05 was attained when olive leaf extract was incorporated into SnO at 500 mg kg^{-1}, but the effect in SbO was less pronounced, with a PF of 1.84 [26]. Other studies on herb-enriched SnO provided values of 1.07 to 1.48 [27]. Moreover, the addition of sage extracts in SnO was shown to give a PF of 1.70, at an enrichment level of 800 mg kg^{-1} [28]. Likewise, a PF level as high as 2.27 was achieved upon the addition of gallic acid in SnO at a content of 200 mg kg^{-1}, but rutin added at the same content was more effective in corn oil, providing a PF of 2.40 [29].

In general, results from the Rancimat test evidencing oil stabilization have been positively correlated with the total polyphenol content of oils [30]. On the other hand, in several instances mentioned above, a PF < 1 evidenced the expression of prooxidant effects of polyphenol-containing extracts, depending on the level of enrichment. In the case examined herein, it was clearly shown that the level of enrichment achieved by the incorporation of the triacetin extract into SnO, SbO, and CnO in no case resulted in increased oil oxidizability, but to the contrary, all oils tested displayed increased stability.

3.3. Formation of Primary and Secondary Lipid Peroxidation Products

To shape a more integrated picture regarding the effect of WOP extract against oil oxidative deterioration, the enriched oils were maintained for 58 days at 60 °C, to simulate long-term stability. At the end of the treatment period, samples were tested for stability by determining primary (PV) and secondary (TBARS) oxidation indices. It should be noted that peroxides are labile compounds and after a certain period may decompose, as opposed to carbonyl compounds, determined by the TBARS assay, which tend to accumulate as oil oxidation progresses. Thus, to verify that 58 days was an appropriate time point to reveal differences among the samples tested, a time course of peroxide accumulation was initially carried out. It can be seen in Figure 3 that after 58 days of treatment at 60 °C, no decrease in PV was observed for any sample tested. Therefore, this time was selected as the end point of the treatment.

The addition of WOP extract in SnO resulted in a protective effect, giving almost an 12% lower PV (Table 4). A similar trend was seen for the enrichment of SbO and CnO, but the differences in PVs found were approximately 2.5 and 4.3%, which fell within the limits of statistical error ($p > 0.05$). Similarly, PVs determined for SnO, SbO, and CnO with the addition of BHT were 15.7, 6.7, and 10% lower compared to the control, respectively. The differences between the protection offered by BHT and WOP were statistically non-significant ($p > 0.05$), which clearly indicated that the use of BHT (a synthetic antioxidant) offered no benefit compared to the natural WOP extract. This is particularly important, considering that the level of several major polyphenols occurring in WOP (Table 1) was much lower than the level of 200 mg kg^{-1} oil used for BHT. A similar outcome has been reported in an earlier investigation, where SnO and SbO containing 200 mg kg^{-1} BHT were equally protected against oxidative deterioration, upon storage at 65 °C, when enriched with WOP ethanolic extracts at a level of 800 mg kg^{-1} oil [31].

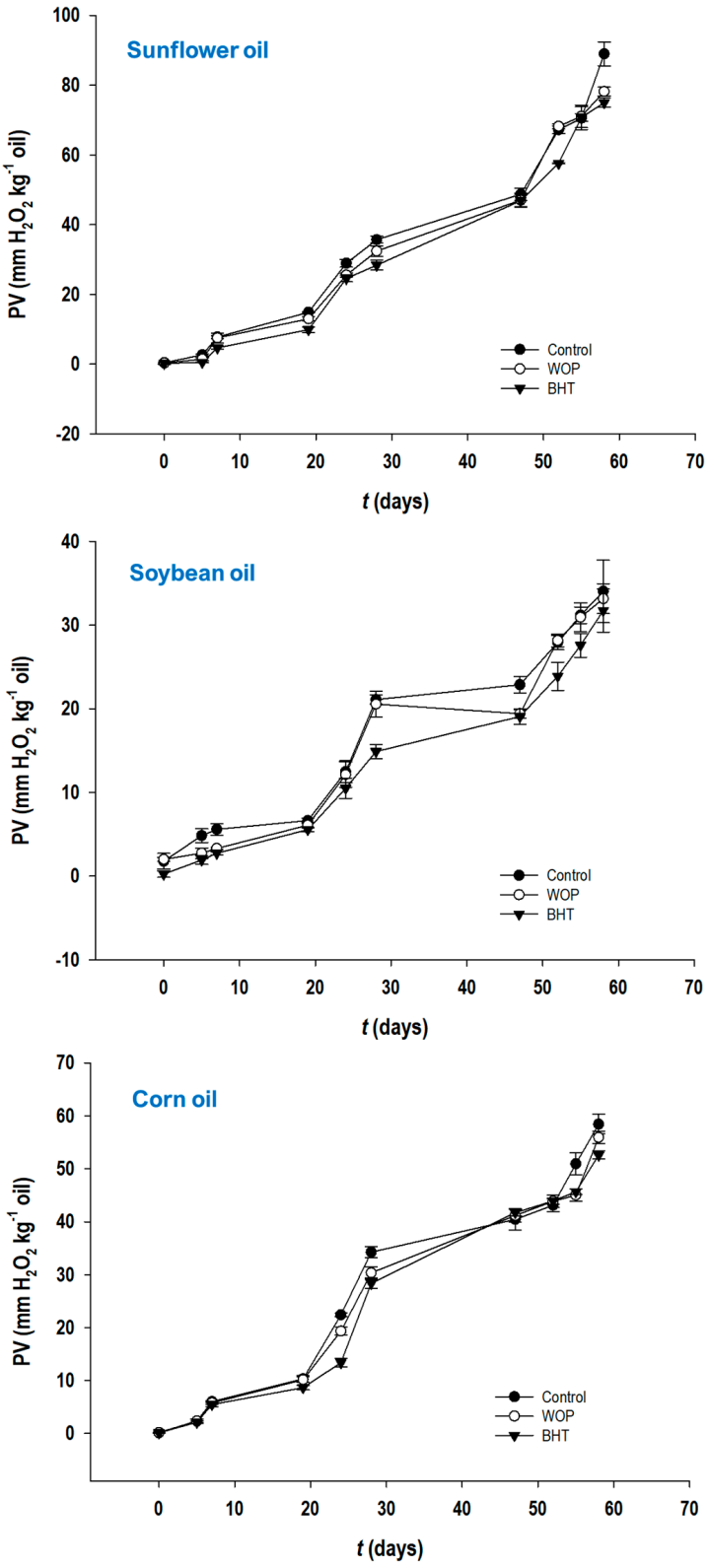


Figure 3. Time course of peroxide value (PV) buildup, in oil samples stored in the dark, at 60 °C, for 58 days. Bars represent standard deviation. Assignments: WOP, oil samples enriched with waste orange peel extract; BHT, oil samples with 200 ppm butylated hydroxytoluene added.

Table 4. Primary and secondary oxidation products determined after maintaining oil samples for 58 days at 60 °C.

Sample	PV (mm H_2O_2 kg^{-1} Oil)	TBARS (mm MDA kg^{-1} Oil)
SnO	88.96 ± 3.45	2.89 ± 0.05
SnO + WOP	78.16 ± 1.29	3.34 ± 0.30
SnO + BHT	74.99 ± 3.33	2.81 ± 0.06
SbO	34.03 ± 3.75	9.56 ± 0.10
SbO + WOP	33.17 ± 1.78	9.29 ± 0.82
SbO + BHT	31.75 ± 2.58	8.85 ± 0.95
CnO	58.44 ± 1.84	3.91 ± 0.42
CnO + WOP	55.92 ± 1.19	3.82 ± 0.44
CnO + BHT	52.59 ± 4.55	4.47 ± 0.52

With reference to TBARS, SnO enriched with WOP exhibited a 15.5% higher value, evidencing a prooxidant effect (Table 4). By contrast, the addition of WOP to SbO and CnO yielded TBARS values which were 2.8 and 2.3% lower compared to the control, respectively, although this was statistically non-significant ($p > 0.05$). The differences in TBARS observed for the samples containing BHT were 2.8 and 7.4%, for the SnO and SbO, respectively, which were also statistically non-significant ($p > 0.05$), suggesting that both WOP and BHT exerted virtually similar protection. On the other hand, the addition of BHT in CnO showed a prooxidant effect, since the BHT-enriched sample had a 17% increased TBARS value.

Because these results showed some discrepancies concerning the actual effect of WOP addition in all three oils tested, the TOTOX value was also considered, to have a deeper insight into the oxidation status of the oils at the end of the examination period. The TOTOX value was determined as follows [32]:

$$\text{TOTOX} = 2\text{PV} + p\text{-AV} \quad (6)$$

The outcome of this approach (Figure 4) confirmed that the WOP extract had a virtually similar effect with the addition of BHT, except for with SnO, for which the protection of WOP extract against oxidation was statistically significant ($p < 0.05$).

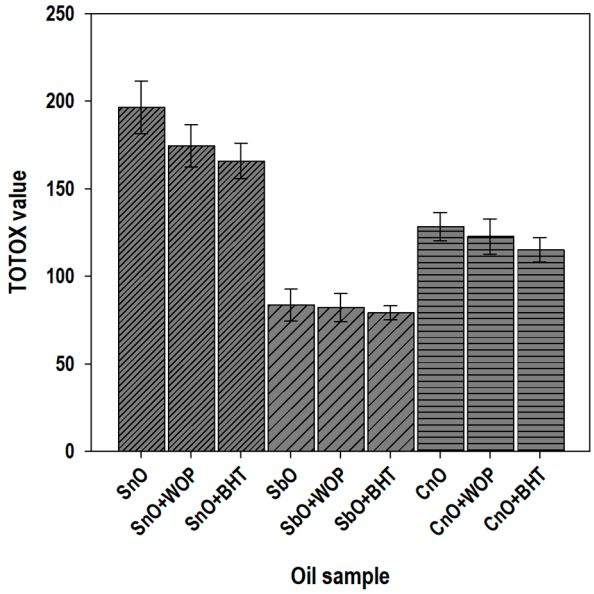


Figure 4. TOTOX value of the oil samples tested. Assignments: WOP, oil samples enriched with waste orange peel extract; BHT, oil samples with 200 ppm butylated hydroxytoluene added. Bars on the columns represent standard deviation. The differences between samples represented by columns of the same filling were statistically non-significant ($p > 0.05$).

Compared to Rancimat, the determination of primary and secondary oxidation products gives additional information regarding the long-term stability of oils. This is particularly important to assess the effect of WOP, since in some instances, the onset of the PV did not correlate with the PF estimated from the Rancimat [32]. Thus, considering the overall picture acquired by both the Rancimat and the long-term stability assays, it could be safely argued that the incorporation of WOP extract in any of the oils tested did not provoke adverse effects with respect to the oxidative stability, but on the contrary, a protective activity was seen. Such an outcome is particularly important in the light of previous examinations, which demonstrated prooxidant effects in vegetable oils enriched with other materials, such as tomato processing by-products [33].

In general, the addition of WOP extracts in seed oils such as sunflower oil [31,34] and soybean oil [31] has been shown to enhance oil stability, at levels higher than 1200 mg kg^{-1} oil. However, these levels were significantly higher than the level of enrichment achieved in this study (36.87 mg kg^{-1} oil). This fact pointed out that, with the methodology proposed herein, seed oils may be fortified with natural polyphenols without compromising their oxidative stability. To the contrary, this fortification might result in a slight protection against rancidity, comparable to that brought about by the addition of the highly effective, synthetic BHT.

3.4. Changes in Antiradical Activity

The Trolox-equivalent antiradical activity (TEAC) of the samples was measured at the beginning and at the end of the treatment, to examine whether changes in the oxidative status of oils could impact their radical-scavenging potential. For SnO, the changes found were statistically non-significant ($p > 0.05$), but for SbO and CnO, the levels of TEAC in the WOP-enriched samples were virtually equal to the control samples (Figure 5). Samples with BHT added exhibited a higher TEAC, yet this difference was not statistically significant. This finding suggested that the oxidation of oils negatively affected their antiradical activity. Considering that the radical-scavenging agents in oils are naturally occurring tocopherols [35,36], but also the phenolic compounds (in this case either WOP polyphenols or BHT), then it could be supported that the depletion of these substances might be responsible for the observed drop in TEAC. On the other hand, this drop in TEAC could also be explained by the accumulation of prooxidant substances, such as peroxides. Therefore, the integration of all these changes might have provoked decreases in TEAC.

The total antioxidant capacity of edible vegetable oils accounts for potential physical and chemical interactions between oil components as well as the integrated effect of the complex variety of antioxidant components against oxidation events in these oils. As a result, qualities like the bioactivity and oxidative stability of edible oils, as well as the total antioxidant capacity, may be indicators of their quality [37]. The inherent radical-scavenging ability of seed oils could be attributed to their content of lipophilic antioxidants, such as tocopherols, which naturally occur in these products [38], but also to their polyphenolic content [39].

Thus, the enrichment of oils with WOP extract would be normally anticipated to enhance the ability of oils to scavenge radicals, due to the presence mainly of the flavonoid fraction of the extract, which is composed primarily of flavanones and flavones (Table 1). The flavonoid fraction represents, except for naringenin and narirutin, which are glycosides, the less polar fraction of WOP extract. This fraction has been shown to express the strongest antiradical activity [40,41] compared to more polar extracts of WOP. These effects may be most probably attributed to flavones, which are powerful antioxidants [42]. However, the scavenging of lipid peroxide radicals by WOP flavonoids would inevitably lead to their depletion, due to peroxide-flavonoid reactions, and thus, after the elapse of a certain period and depending on the conditions (e.g., light, temperature), the enriched oils would be eventually stripped of antioxidant protection [43]. This would be presumably reflected in the TEAC, and this was most probably the reason for the drop in the radical scavenging ability observed towards the end of the examination period. The fact that secondary oxidation

products were accumulated, as revealed by the TBARS and *p*-AV assays, evidenced the manifestation of such phenomena.

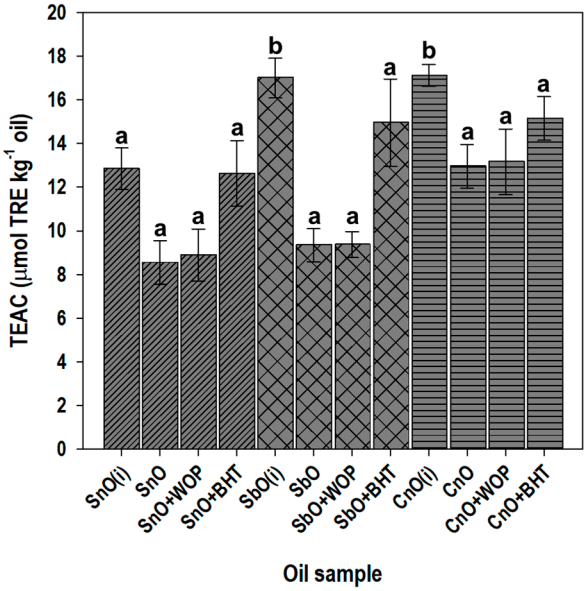


Figure 5. Trolox-equivalent antiradical activity (TEAC) of the oil samples tested. Assignments: WOP, oil samples enriched with waste orange peel extract; BHT, oil samples with 200 ppm butylated hydroxytoluene added. Bars on the columns represent standard deviation. Columns with the same fill denoted with different letters (a, b), display statistically different values ($p < 0.05$).

4. Conclusions

The study reported herein was an attempt to generate polyphenol-enriched extracts from WOP, using an innovative extraction process based on ethanol/triacetin mixtures, which is heretofore unreported. By employing this technique, three commonly consumed commercial oils were enriched with polyphenols from WOP, and then tested for stability. The results drawn showed that, although the enrichment level was low, the oil samples exhibited stability comparable to the oil samples with the synthetic antioxidant BHT added to them. However, in some instances, neither WOP extract nor BHT afforded significant protection, as was revealed by comparison with the control samples. In all cases, the antiradical activity of the oil samples tested showed a decline at the end of the examination period, which might be attributed to oxidation product accumulation, antioxidant depletion, or both. It is recommended that further research is required to increase the level of enrichment, in order to achieve a higher degree of protection against the oxidation of oils. This will highlight the importance of WOP as a source of natural oil antioxidants, which could effectively replace the synthetic ones. Furthermore, the results of the study highlighted WOP as a valuable bioresource for the recovery of high-value-added compounds, such as food antioxidants. Future investigations should demonstrate the applicability of such extracts in foods but also in other commodities (e.g., cosmetics). Such studies would pave the way for the large-scale valorization of WOP as an abundant and cost-effective natural constituent, and could comprise part of biorefinery approaches to establishing strategies towards wider circular economy policies.

Author Contributions: Conceptualization, D.P.M.; methodology, D.K., D.P., V.A. and T.C.; investigation, D.K., D.P., V.A. and T.C.; resources, D.P.M. and S.I.L.; data curation, D.K., D.P., V.A. and T.C.; writing—original draft preparation, D.K., D.P.M. and S.I.L.; writing—review and editing, D.P.M. and S.I.L.; supervision, D.P.M. and S.I.L.; project administration, D.P.M. and 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: The data will be available on request.

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

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