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Use of Amberlite Macroporous Resins To Reduce Bitterness in Whole Olives for Improved Processing Sustainability

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ABSTRACT: Olives are inedible because of high levels of bitter phenolics (e.g., oleuropein) which are removed during commercial olive processing. Current commercial processing methods are highly water-intensive, produce toxic wastewater, and are environmentally unsustainable. To address this, macroreticular polymeric resins were used to assist debittering and decrease water use. Amberlite resins XAD4, XAD16N, XAD7HP, and FPX66 were evaluated for the ability to adsorb bitter and/or high-value phenolic compounds (i.e., oleuropein, ligstroside, oleuropein aglycone, ligstroside aglycone, oleocanthal, oleacein, and hydroxytyrosol) from whole olives during typical brine storage. All resins effectively adsorbed oleuropein and ligstroside. FPX66 reduced oleuropein in whole olives suspended in a 1.0% acetic acid brine to 0.635 mg/kg wet weight in 2.5 months with no further processing. This concentration is below levels measured in commercial California-style black ripe olives (0.975 mg/kg wet weight). Resins in storage brines effectively decrease levels of bitter phenolic compounds without additional lye processing. Excellent recoveries of high-value phenolic compounds are obtained from resins (e.g., 80.2 ± 3.3% to 89.4 ± 8.9% hydroxytyrosol).

KEYWORDS: *table olives, Amberlite macroporous resins, FPX66, oleuropein, ligstroside, debittering*

INTRODUCTION

Table olives, fruits of the *Olea europea* L. drupe, are a popular food consumed worldwide. Olive oil and table olives are essential components of the Mediterranean diet, a diet linked to reducing cardiovascular disease,[1] Alzheimer's disease,[2] and other morbid health conditions related to aging.[3] The health-promoting properties of olives are attributed to a phenolic composition that is unique to *Olea europaea* L.;[4,5] these phenolic compounds exhibit a range of antioxidant,[6] anti-inflammatory,[7,8] anticancer,[9,10] antimicrobial,[11,12] and antiviral properties.[10]

Phenolic compounds unique to olives include the secoiridoids, a subclass of iridoids derived from the cleavage of the cyclopentane ring at the 7,8-carbon bond. Secoiridoids are secondary plant metabolites that accumulate in the flesh and skin of maturing olive fruit and are generally regarded as a chemical defense against herbivores and pathogens because iridoid glycosides generally have a bitter taste and have antifeedant and growth inhibitory activities against insects.[13,14] The most abundant secoiridoids in olive fruit include oleuropein, demethyloleuopein (in mature fruit of some cultivars), and ligstroside whereas nüzhenide and nuzhenide oleoside are present in lyophilized olives and olive seeds.[15−18] Secoiridoids in olive fruit are susceptible to hydrolysis (e.g., hydrolysis via β-glucosidase, esterases) and acid/base-catalyzed degradation during maturation and storage and products include oleuropein aglycone, ligstroside aglycone, oleocanthal, oleacein, hydroxytyrosol, and tyrosol.[15,18] (Figure 1).

Oleuropein, a highly bitter compound, is the most abundant phenolic compound present in most olive cultivars at harvest and can reach 140 mg g^{-1} on a dry matter basis in young olives[19] and up to 20 mg g^{-1} are reported for olives of several cultivars at harvest stage.[20] For some olive cultivars, the

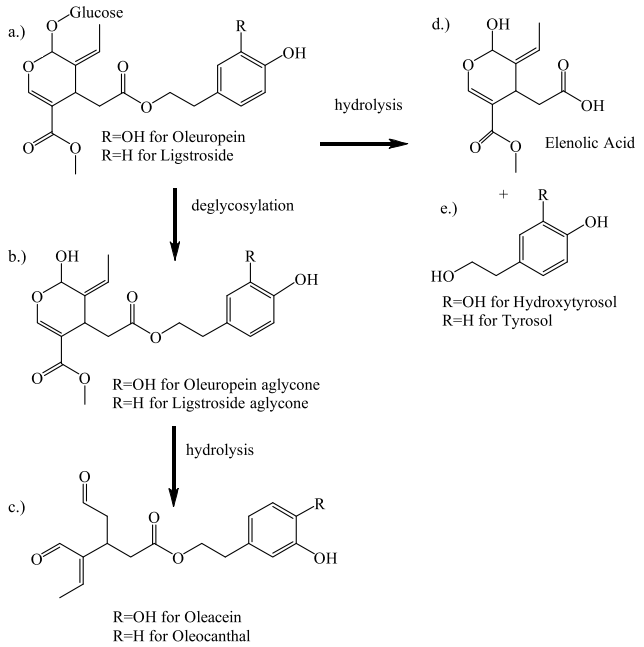

Figure 1. Phenolic compounds related to olive bitterness: (a) R = H ligstroside, R = OH oleuropein; (b) R = H ligstroside aglycone, R = OH oleuropein aglycone; (c) R = H oleacein, R = OH oleocanthal; (d) elenolic acid, (e) R = H tyrosol, R = OH hydroxytyrosol.

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concentration of demethyloleuropein can be greater than that of oleuropein at harvest. Although oleuropein is considered the primary bitter compound in olives, ligstroside, oleuropein aglycone, ligstroside aglycone, oleocanthal, and oleacein also correlate with olive bitterness.[21] The levels of oleuropein must be significantly reduced through processing or curing to make olives edible. Traditional processing methods rely on the hydrolysis of oleuropein and ligstroside into nonbitter products (i.e., hydroxytyrosol, tyrosol, etc.).[21]

Although levels of phenolic compounds must be reduced to make olives edible, there is an economic incentive for recovering olive phenolics as value added ingredients or supplements. Oleuropein exhibits effective anticancer[9,10] and antimicrobial activity,[11,12] whereas oleocanthal is a potent anti-inflammatory agent that exhibits properties similar to ibuprofen.[8] In addition, oleacein, hydroxytyrosol, oleuropein, and oleuropein aglycone all exhibit strong antioxidant activity.[8,9,22] These phenolic compounds are highly bioavailable with a 55−60% demonstrated uptake of ligstroside aglycone, oleuropein aglycone, hydroxytyrosol, and tyrosol.[23,24] Isolated hydroxytyrosol demonstrates antioxidant and anti-inflammatory effects, and the European Food Safety Authority (EFSA) Panel considers that to bear the claim referring to the protection of blood lipids from oxidative damage, 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) should be consumed daily.[10]

Despite the health and economic value of olive phenols, current commercial table olive processing methods rely on the removal of oleuropein and ligstroside through acid/base and or enzymatic hydrolysis and the phenolic products are not recovered.[25] Today, there are three main commercial approaches used for debittering olives. These include the following: Greek natural, Spanish green, and California-style black ripe processing methods. Each method of debittering produces a product with unique texture, chemical, and sensory profiles.[26] Olives produced using the California-style black ripe method result in the lowest levels of total phenolics as well as lowest mean concentrations of oleuropein (0.975 mg/kg wet weight) and hydroxytyrosol (19.981 mg/kg wet weight).[26,27]

Table olive processing methods are some of the most water-intensive processing methods used in commercial food industry. For example, processing California-style black ripe olive requires up to 8.0 m^3/t of olive, and of this, 2.0 m^3/t becomes a lye wastewater fraction that must be treated and/or disposed of in evaporation ponds.[28] Commercial Spanish olive processing methods are also water-intensive, requiring 3.9−7.5 m^3/t of olive.[29] Greek style processing methods are less water-intensive using 0.9−1.9 m^3/t of olive.[29] Wastewater produced through olive processing is characterized by a high chemical oxygen demand (COD) value and is considered toxic to plant, microbial, and animal life.[30] The wastewater is high in phenolics, sodium chloride, sugar, and other compounds that contribute to a high organic burden.[30−32] Global climate change has increased serious drought conditions and pressure on water use in California. In this new climate, novel low-water methods that generate less toxic wastewater for debittering table olives are desirable.

One solution for reducing the organic burden of olive processing wastewater is by filtering the effluent with Amberlite macroporous resins.[33−35] Resins are reusable and stable and have been used to adsorb phenolics from a variety of products including flavonoids from Ginkgo biloba,[36] anthocyanins from grape pomace extracts,[37] polyacetylenes from carrot juice,[38] antioxidants from blueberries,[39] and phenolics from olive mill wastewater.[39−41] Amberlite macroporous resins have demonstrated the ability to specifically adsorb hydroxytyrosol[40] and also tyrosol and oleuropein from olive mill wastewater.[41] However, this approach has yet to be applied to the reduction of oleuropein and ligstroside and other bitter phenolics in whole olives for the express purpose of debittering.

Macroporous cross-linked nonionic Amberlite resins XAD4, XAD16N, XAD7HP, and FPX66 have shown the greatest potential in adsorbing olive phenolics.[34,35,42] These resins are sold as small white translucent beads that have both a continuous polymer phase and a continuous pore phase with high surface area and porosity. They operate well in a wide pH range (0−14) and with high physical, chemical, and thermal stability.[43−46] Phenolic adsorption by resins is attributed to a combination of multiple interactions including hydrophobic interactions, hydrogen bonding, and electrostatic interactions.[47,48] Debittering olives using resins, especially during storage in brines, would have many benefits, including a reduction in the use of water and lye during processing, increasing industry sustainability, and decreasing the amount and toxicity of processing wastewater. In addition, adsorbed phenolics can be recovered from resins as value-added ingredients.

Herein, XAD4, XAD16N, XAD7HP, and FPX66 resins were evaluated for their ability to remove phenolics from whole olives during normal brine storage, thereby decreasing the need for excess lye processing, reliance on water, and generation of toxic wastewater.

MATERIALS AND METHODS

Chemicals and Reagents. Oleuropein and tyrosol (2-(4-hydroxyphenyl)ethanol) were purchased from Sigma-Aldrich (St. Louis, U.S.A.). Hydroxytyrosol was purchased from Indofine (Hillsborough, NJ, USA). High-performance liquid chromatography (HPLC) grade acetic acid, acetonitrile, and methanol were purchased from Fisher Scientific. Oleacein (decarboxymethyl oleuropein aglycone), oleocanthal (decarboxymethyl ligstroside aglycone), ligstroside aglycone, and oleuropein aglycone were isolated from Thassos olives according to the previously described method.[49]

Pretreatment of Resins. XAD4, XAD16N, and XAD7HP (Sigma-Aldrich) and FPX66 (Dow Chemical, Midland, MI, U.S.A.) resins were suspended in 100% methanol and manually stirred with a glass stirring rod for 30 min. Resins were separated from methanol via a Buchner funnel (Whatman No. 10 filter paper) and washed with three loading volumes of water.

Olive Extract. Seventy green Manzanilla olives harvested in the fall of 2015 and stored in a 1.0% acetic acid brine for 5 months were removed from the brine, pitted, and blended in 1 L of deionized water. Solid material was separated out via a Buchner funnel (Whatman No. 10 filter paper) and resulting liquid brought up to a volume of 2 L with deionized water. A 40 mL aliquot of olive extract was stored in a capped polypropylene centrifuge tube and frozen immediately at −80 °C until analysis.

Adsorption of Phenolics to Resins. Five grams (5 g) of pretreated hydrated resin (i.e., XAD4, XAD16N, XAD7HP, or FPX66) was combined with 40 mL of olive extract in a 50 mL polypropylene sterile centrifuge tube. A control sample of 40 mL of olive extract was placed in a centrifuge tube with no resin. Tubes were sealed and placed in a gyrotory water bath shaker (Model G76 New Brunswick Scientific Co., Edison, NJ, U.S.A.) at 25 °C and shaken at a rate of approximately 240 rpm for 16 h. After exposure, resin was separated from extract using Whatman No. 10 filter paper. Extracts were performed in triplicate. The phenolic concentration was quantified using UHPLC-ESI (MS/MS) in time-zero extracts, resin-

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treated extracts, and control untreated extracts according to a previously established method.[50]

Passive Adsorption of Phenolics to Resins. A 5 g sample of pretreated resin (i.e., FPX66, XAD4, XAD16N, or XAD7HP) was mixed with 40 mL of olive extract and placed in a 250 mL Erlenmeyer flask at 25 °C. The control contained 40 mL of olive extract and no resin. Flasks were not sealed and were swirled by hand between sampling. A 1 mL aliquot of supernatant was sampled after 4, 10, 16, 20, and 30 min. Replicate samples were taken at each time point and the phenolic concentration quantified using UHPLC-ESI (MS/MS).[50]

Resin-Assisted Olive Debittering. Olives obtained from Musco Olive Company were harvested on October 27, 2015, and shipped that day at 25 °C from Tracy, California, to Davis, California. A selection of raw olives was frozen on day 0 and stored in −80 °C until sampled. Olives were treated with FPX66 resin by placing 15 unblemished green whole olives in 125 mL Erlenmeyer flasks with 25 g of activated FPX66 resin and 60 mL of 1.0% acetic acid in deionized (DI) water (pH ∼4). Controls were created by placing 15 unblemished green whole olives in 125 mL flasks with 60 mL of 1.0% acetic acid in DI water (pH ∼ 4). Flasks were sealed until sampling. Olives were sampled on days 0, 6, 26, 76, and 273. Olives, brine, and resin were separated using a Buchner funnel and Whatman No. 10 filter paper. Olives were separated into three composite samples of 5 olives each, weighing approximately 26 g (wet weight). Composite samples were blended (Waring WSG30 Commercial Spice Grinder-120 V) and placed in a 50 mL conical tube. Lipids were removed with three successive 10 mL aliquots of hexane. Tubes were shaken vigorously for 1 min and centrifuged at 4000 rpm for 5 min. The lipid layer was decanted and the defatted pulp frozen at −80 °C for 12 h. Samples were then freeze-dried to a consistent weight, and the resulting powder was sieved through a Tyler standard #65 screen with a 0.210 mm opening. Compounds were extracted with a 1:40 w/v of 60% methanol in water, with 1 min of agitation and centrifugation at 4000 rpm for 5 min. Brine was sampled directly without extraction. Olive extracts and brine were filtered through a 0.22 μm nylon filter prior to UHPLC-ESI MS/MS analysis. Samples were diluted to be within the linear dynamic range.

Phenolic Desorption and Recovery from Resin. Phenolic compounds adsorbed onto the resin were desorbed by solvent extraction at a ratio of 1 g of resin to 5 mL of 100% ethanol. Ethanol was chosen as it demonstrates high recovery of olive phenolics from resins.[40]

Ultra-High-Performance Liquid Chromatography-Electron Spray Ionization Tandem Mass Spectrometry (UHPLC-(ESI) MS/MS). UHPLC analysis was performed according to the previously described method.[44] Briefly, compounds were analyzed on an Agilent 1290 Infinity ultra-high-performance liquid chromatography system (UHPLC) interfaced to a 6460 triple-quadrupole mass spectrometer (MS/MS) with electrospray ionization (ESI) via Jet Stream technology (Agilent Technologies, Santa Clara, CA, USA). The UHPLC was equipped with a binary pump with an integrated vacuum degasser (G4220A), an autosampler (G4226A) with thermostat (G1330B), and a thermostated column compartment (G1316C). Compounds were separated using a Poroshell 120 C_{18} column (3.0 × 50 mm, 2.7 μm, Agilent Technologies). The mobile phase consisted of a linear gradient, flowing at 0.7 mL/min, of 0.01% acetic acid in purified water (A) and 0.01% acetic acid in acetonitrile (B) as follows: 10% B, 0−2 min; 10−30% B, 2−3 min; 30−65% B, 3−5 min. The column temperature was 20 °C, and the injection volume was 5 μL. Oleuropein, oleuropein aglycone, ligstroside, ligstroside aglycone, hydroxytyrosol, tyrosol, oleocanthal, and oleacein were quantified against purified standards. Ligstroside was quantified using relative quantification against oleuropein standards.

Test of Significance. To determine if a significant decrease in concentration occurred with resin treatments, Student *t*-tests were conducted. The *t*-test was two-tailed, and two-sampled assuming unequal variance with a significance value of $\alpha = 0.05$.

RESULTS

Weather extremes are recognized to pose a significant challenge to food systems in recent years and are likely to become even more important in the future. In California, drought, fire, and water use are primary concerns, creating a new paradigm for food manufactures that rely heavily on unrestricted water use for food processing. One such industry is the table olive industry.

Herein, XAD4, FPX66, XAD16N, and XAD7HP (Table 1) were evaluated for the ability to adsorb oleuropein, ligstroside, oleuropein aglycone, ligstroside aglycone, oleocanthal, oleacein, hydroxytyrosol, and tyrosol from whole olives during typical brine storage as a method for decreasing water use in table olive processing.

Table 1. Chemical and Physical Properties of Amberlite Resins[a]

	Resin			
	FPX66	XAD16N	XAD4	XAD7HP
structure	aromatic	aromatic	aromatic	aliphatic
pH range	0−14	0−14	0−14	0−14
max temp	150 °C	150 °C	300 °C	175−210 °C
moisture holding capacity	60−68%	62−70%	54−60%	61−69%
surface area	>700 m^2/g	>800 m^2/g	>750 m^2/g	>380 m^2/g
porosity	>1.4 mL/g	>0.55 mL/mL	>0.50 mL/mL	>0.50 mL/mL

[a]Information obtained from product specification sheets provided by Rohm and Hass.[43−46]

Initially, olive extracts were treated with XAD4, FPX66, XAD16N, and XAD7HP for 16 h to assess the ability of the resins to bind the compounds in olives which are related to olive bitterness. The phenolics were quantified in the olive extracts and in materials recovered from resins using UHPLC-(ESI) MS/MS. These results are reported as percentage remaining after 16 h of resin exposure in Table 2. All resins demonstrated the ability to reduce levels of oleuropein, ligstroside, oleacein, and oleuropein aglycone below the limit of detection at 16 h. Ligstroside aglycone and oleocanthal were below the limit of detection in the initial extracts (i.e., time 0). When compared to initial conditions, XAD4, FPX66, and XAD16N significantly reduced levels of hydroxytyrosol to 15.1−25.7% and tyrosol to 12.2−16.9% of the initial concentration in olive extracts, whereas reduction on the XAD7HP resin was significantly lower for hydroxytyrosol (40.1 ± 7.3% remaining of initial concentration) and tyrosol (26.9 ± 4.3% remaining of initial concentration). XAD7HP is an aliphatic resin with a smaller surface area (>380 m^2g^{-1}) as compared to the aromatic FPX66, XAD4, and XAD16N resins. This may have contributed to the lower adsorption observed on the XAD7HP resin (Table 1). Recoveries of phenolic compounds from the resins were excellent for all phenolics of interest: 66.5−73.1% oleuropein, 68.3−75.3% oleuropein aglycone, 68.0−74.1% ligstroside, 43.0−64.8% oleacein, 89.4−102.5% tyrosol, and 80.2−89.4% hydroxytyrosol.

To evaluate the kinetics of phenolic adsorption to the resins, olive extracts were exposed to resins and sampled at 4, 10, 16, 20, and 30 min. Levels of olive phenolics were quantified in these samples (Figure 2a−e). Levels of oleuropein, ligstroside,

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Table 2. Adsorption and Recovery of Select Phenolic Compounds from Olive Extracts Exposed to Amberlite Resins for 16 h[a]

sample at 16 h	resin-treated extract	recovered from resin
Oleuropein		
control	90.9 ± 1.7%	
FPX66	ND	70.0 ± 2.8%
XAD4	ND	73.1 ± 1.7%
XAD16N	ND	70.9 ± 1.4%
XAD7HP	ND	66.5 ± 6.2%
Ligstroside		
control	96.9 ± 0.5%	
FPX66	ND	70.7 ± 5.5%
XAD4	ND	73.8 ± 0.8%
XAD16N	ND	74.1 ± 2.6%
XAD7HP	ND	68.0 ± 2.8%
Oleacein		
control	100.0 ± 9.3%	
FPX66	ND	58.1 ± 3.3%
XAD4	ND	64.8 ± 7.7%
XAD16N	ND	43.0 ± 6.7%
XAD7HP	ND	55.0 ± 5.9%
Oleuropein Aglycone		
control	100.5 ± 1.5%	
FPX66	ND	68.3 ± 0.8%
XAD4	ND	73.7 ± 4.8%
XAD16N	ND	75.3 ± 1.5%
XAD7HP	ND	72.2 ± 3.0%
Hydroxytyrosol		
control	106.8 ± 6.4%	
FPX66	17.8 ± 4.6%	85.5 ± 1.2%
XAD4	15.1 ± 1.8%	80.2 ± 3.3%
XAD16N	25.7 ± 1.7%	83.7 ± 3.8%
XAD7HP	40.1 ± 7.3%	89.4 ± 8.9%
Tyrosol		
control	114.3 ± 3.1%	
FPX66	14.9 ± 0.3%	89.4 ± 0.6%
XAD4	12.2 ± 0.3%	92.0 ± 10.4%
XAD16N	16.9 ± 0.2%	95.6 ± 8.3%
XAD7HP	26.9 ± 4.3%	102.5 ± 9.3%

[a]Expressed as a percentage of original concentration in olive extract at time 0 min.

and oleacein were reduced to ND−5% of the initial concentration in 10 min on the FPX66, XAD16N, and XAD7HP resins, and in 20−30 min on the XAD4 resin. All resins effectively adsorbed oleuropein, ligstroside, and oleacein by 30 min, demonstrating a high affinity for these compounds. The adsorption of tyrosol and hydroxytyrosol to all resins was slower as compared to that of oleuropein, ligstroside, and oleacein (Figure 2a−e). At 30 min, tyrosol concentration was reduced to only 35−60% initial concentration and hydroxytyrosol to 81−93% initial concentration.

Although adsorption of oleuropein, ligstroside, and oleacein was rapid on all resins, a higher affinity was observed on the FPX66 and XAD16N resins as compared to that on XAD7HP and XAD4. FPX66 and XAD16N are cross-linked aromatic polymers with similar chemical and physical properties, with exception of porosity (Table 1). However, FPX66 resin can be purchased as certified food safe and is currently used in commercial citric juice debittering. Therefore, FPX66 was used to determine if bitterness levels could be reduced in whole olives stored in a typical storage brine (1.0% acetic acid) over 9 months. These are typical conditions that olives are subjected to prior to commercial lye processing of California-style black ripe olives.

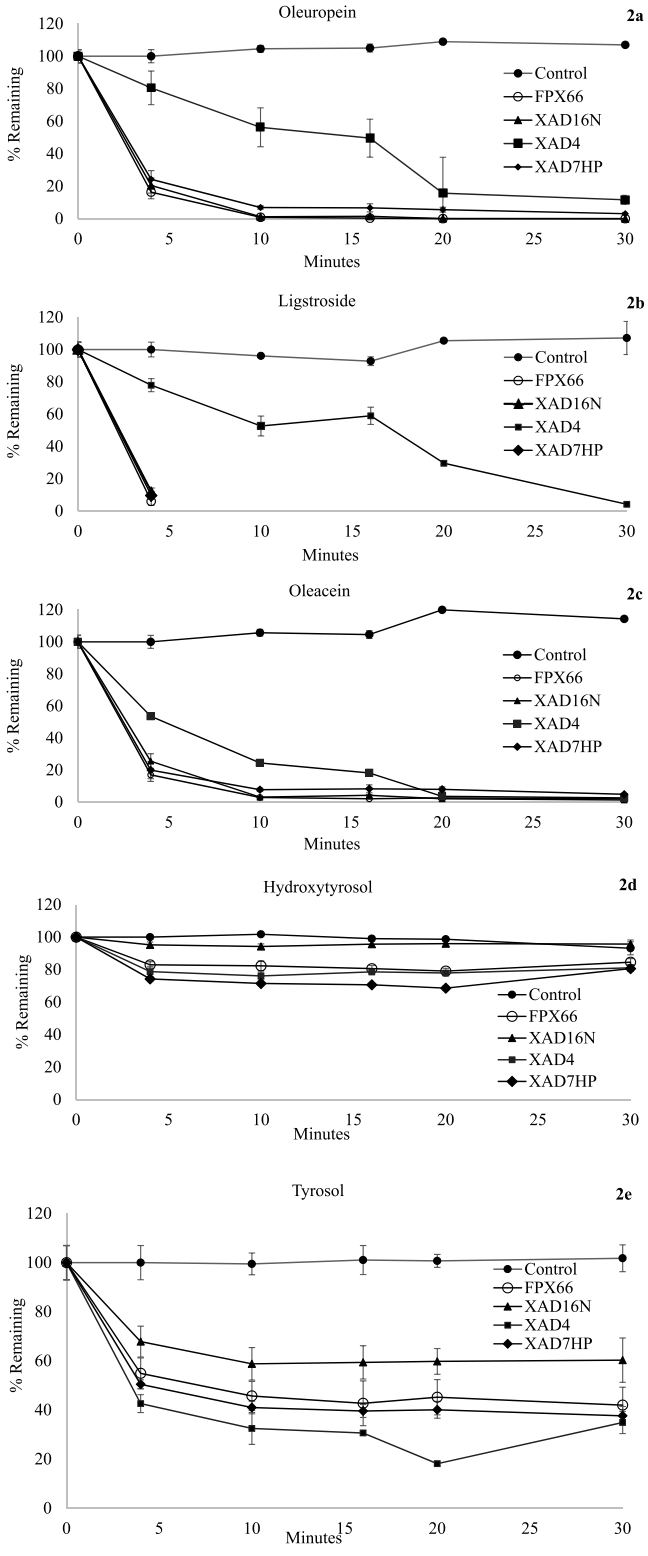

Figure 2. Dynamic changes in phenolic concentration (expressed as % initial concentration) when exposed to Amberlite resins FPX66, XAD16N, XAD7HP, and XAD4 over 30 min: (a) oleuropein, (b) ligstroside, (c) oleacein, (d) hydroxytyrosol, and (e) tyrosol.

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The FPX66 resin was effective at significantly reducing levels of oleuropein, oleuropein aglycone, and ligstroside in whole olives (Table 3). After 76 days of storage with FPX66 resin, oleuropein concentration in whole olives was significantly reduced to 0.635 ± 0.034 mg kg^{-1} olive (wet weight) as compared to that in control olives (19.396 ± 1.676 mg kg^{-1} wet weight). Earlier studies demonstrate that commercial nonbitter California-style black ripe olives have a mean oleuropein concentration of 0.974 mg kg^{-1} olive (wet weight) at time of consumption.[50] Levels of oleuropein were not reduced below 2.502 ± 0.583 mg kg^{-1} olive (wet weight) in olives stored without FPX66 resin. These results indicate that holding olives in a storage brine with FPX66 resin will result in the reduction of oleuropein to edible levels without additional lye processing. The initial levels of ligstroside were low (3.094 ± 0.237 mg kg^{-1} (wet weight) relative to that of oleuropein and were significantly decreased in resin-treated olives (0.003 ± 0.001 mg kg^{-1} wet weight) as compared to those in the controls (0.592 ± 0.043 mg kg^{-1} wet weight). Ligstroside is below ± the limit of detection in California-style black ripe olives.[50] Oleuropein aglycone concentrations in both control (3.773 ± 0.800 mg kg^{-1} wet weight) and treated olives (2.884 ± 1.561 mg kg^{-1} wet weight) were significantly reduced relative to initial levels (116.778 ± 5.183 mg kg^{-1} wet weight) after just 6 days of storage. By day 76, oleuropein aglycone was no longer detected in resin-treated olives and was detected at a concentration of 0.351 ± 0.029 mg kg^{-1} (wet weight) in the control, which is significantly higher than the measured oleuropein aglycone concentration of 0.003 mg kg^{-1} (wet weight) in California-style black ripe olives.[50,51]

Hydroxytyrosol concentration decreased in both control and resin-treated olives. The concentration of hydroxytyrosol was significantly lower (α = 0.05) in resin-treated olives at 6, 26, and 76 days, whereas no significant difference was observed on day 273 (Table 3). In contrast, the levels of tyrosol increased in both the control and resin-treated olives over time (Table 3).

Table 3. Influence of FPXX Amberlite Resin on Concentrations of Select Phenolics Compounds in Olives Stored in Acidic Brine for 9 Months

compound	day	control olives, mg kg^{-1} olive (wet weight)	treated olives, mg kg^{-1} olive (wet weight)
oleuropein	0	83.401 ± 4.433	83.401 ± 4.433
	6	85.863 ± 15.251	37.521 ± 2.974
	26	40.450 ± 2.385	12.150 ± 3.096
	76	19.396 ± 1.676	0.635 ± 0.034
	273	2.502 ± 0.583	0.335 ± 0.004
ligstroside	0	3.094 ± 0.237	3.094 ± 0.237
	6	1.837 ± 0.368	2.016 ± 0.778
	26	0.943 ± 0.086	0.244 ± 0.080
	76	0.592 ± 0.043	0.003 ± 0.001
	273	0.639 ± 0.041	ND
oleuropein aglycone	0	116.778 ± 5.183	116.778 ± 5.183
	6	3.773 ± 0.800	2.884 ± 1.561
	26	2.273 ± 0.087	0.635 ± 0.186
	76	0.351 ± 0.029	ND
	273	ND	ND
hydroxytyrosol	0	56.253 ± 2.069	56.253 ± 2.069
	6	33.321 ± 1.233	28.351 ± 0.235
	26	23.329 ± 0.848	20.673 ± 0.636
	76	26.170 ± 4.495	10.460 ± 1.854
	273	11.390 ± 4.952	16.091 ± 1.616
tyrosol	0	1.851 ± 0.124	1.851 ± 0.124
	6	11.345 ± 0.495	11.795 ± 1.762
	26	13.137 ± 0.893	9.322 ± 0.977
	76	14.464 ± 1.639	6.102 ± 0.367
	273	22.057 ± 2.241	9.512 ± 0 0.287

Table 4. Concentrations of Select Phenolics Compounds in the Acidic Brines of Control Olives and in Acidic Brines and Recovered from Resins of Olives Exposed to FPXX Amberlite Resin, over 9 Months of Storage[a]

			resin-treated olives	
compound	day	control olives brine	brine	resin
oleuropein	6	8.623 ± 2.644%	0.019 ± 0.0002%	21.253 ± 0.074%
	26	7.700 ± 0.491%	0.009 ± 0.003%	19.221 ± 2.009%
	76	4.903 ± 0.331%	0.024 ± 0.004%	14.319 ± 0.558%
	273	4.520 ± 0.017%	0.004 ± 0.006%	10.061 ± 0.882%
ligstroside	6	28.674 ± 0.008%	ND	13.337 ± 2.207%
	26	8.112 ± 0.009%	ND	11.214 ± 1.812%
	76	0.841 ± 0.010%	ND	8.104 ± 2.516%
	273	3.011 ± 0.005%	ND	4.622 ± 1.071%
oleuropein aglycone	6	0.725 ± 0.00003%	ND	18.249 ± 1.795%
	26	0.843 ± 0.0002%	ND	19.608 ± 5.087%
	76	0.216 ± 0.0002%	ND	9.549 ± 0.373%
	273	0.066 ± 0.00008%	ND	1.229 ± 0.349%
hydroxytyrosol	6	59.500 ± 1.665%	41.655 ± 4.442%	16.990 ± 0.005%
	26	70.711 ± 2.235%	44.794 ± 2.639%	20.212 ± 0.293%
	76	76.824 ± 2.930%	65.473 ± 2.513%	31.739 ± 4.672%
	273	89.890 ± 3.805%	66.216 ± 1.849%	28.134 ± 2.114%
tyrosol	6	293.121 ± 14.147%	7.580 ± 0.588%	24.142 ± 4.825%
	26	403.148 ± 21.719%	11.271 ± 0.576%	27.725 ± 0.648%
	76	602.934 ± 34.188%	26.093 ± 14.839%	60.930 ± 20.260%
	273	730.979 ± 27.727%	35.330 ± 9.429%	66.706 ± 14.634%

[a]Concentrations are expressed as a percentage of original concentration in initial olives at time 0 min.

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Hydroxytyrosol is generated from the hydrolysis of oleuropein and oleuropein aglycone, just as tyrosol is generated from the hydrolysis of ligstroside and ligstroside aglycone.[52] However, hydroxytyrosol undergoes spontaneous oxidation and polymerization because of the ortho diphenol functional group on hydroxytyrosol.[17] This explains the increase in tyrosol and decrease in hydroxytyrosol during olive storage.

Levels of oleuropein, ligstroside, oleuropein aglycone, hydroxytyrosol, and tyrosol measured in the brines on days 6, 26, 76, and 273, expressed as a percentage of the original molar content at time 0, are given in Table 4. Oleacein, ligstroside aglycone, and oleocanthal were below the limit of detection in the control and treatment brines and resin at all time points. In the control brine, 4.5−8.6% of the oleuropein detected in initial olives ($t = 0$) was recovered in the brine. In comparison, only 0.004−0.024% of the oleuropein was recovered in the brine of treated olives (Table 4). Ligstroside and oleuropein aglycone were below the limit of detection in the brine of the treated olives whereas 0.841−28.674% and 0.066−0.843% were recovered in the brine of the control olives, respectively (Table 4). Hydroxytyrosol concentrations were relatively high in the control brines (59.500−98.890% of initial) whereas concentrations in the treated brine were significantly lower (41.655−66.216% of initial). Levels of tyrosol increased with storage time in both the control and resin-treated brines; however, levels were relatively higher (293.121−730.979% of initial) in control brines (Table 4).

The levels of all phenolics measured in the brines of the resin-treated olives were significantly lower than levels detected in the control. This indicates that olives stored with resins will produce storage brine wastewater that is significantly lower in phenolics, lowering the COD and toxicity of the waste effluent.

To date, the recovery of high-value phenolics from brine storage wastewater has not been evaluated. However, phenolics such as hydroxytyrosol and oleuropein have potent biological activity. Recovery of these phenolics from the resins used to debitter olives passively during storage could provide an additional stream of value-added ingredients for use in other products (supplements, cosmetics, etc). Herein, we demonstrate that the phenolics adsorbed onto resins during brine storage are easily recovered from resins using ethanol (Table 4). Recoveries of oleuropein, ligstroside, and oleuropein aglycone decreased with time, likely due to hydrolysis, polymerization, and oxidation reactions. Conversely, levels of hydroxytyrosol and tyrosol increased with storage time, again reflecting the hydrolysis of oleuropein and ligstroside.

The data demonstrate that higher yields of oleuropein, ligstrostride, and oleuropein aglycone will be achieved by extracting resins during early stages of brine storage, while their hydrolysis products will be recovered in higher concentration at later time points.

The results of this study demonstrate the feasibility of using Amberlite macroporous resins suspended in storage brines to reduce the concentration of bitter phenolics in whole olives passively during storage. This promising new technology has the potential to reduce water usage during table olive processing, reduce toxicity of brine wastewater, and provide an opportunity for recovery of high-value olive phenolics as a second stream of revenue for olive producers. Future work should focus on investigating the influence of salt, pH, storage agitation, and oxidation on the resin adsorption process. Additionally, sensory studies will help to determine how resin-assisted debittering impacts consumer perception of texture, flavor, and color of cured table olive products.

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ABBREVIATIONS USED

MS, mass spectrometry; UHPLC, ultra-high-performance liquid chromatography; ESI, electron spray ionization; MS/MS, triple quadrupole mass spectrometer

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