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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., $80.2 \pm 3.3\%$ to $89.4 \pm 8.9\%$ hydroxytyrosol).

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

INTRODUCTION

Table olives, fruits of the *Olea europaea* 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, demethyloleuropein (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 olives¹⁹ 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 demethyleuropein 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 m³/t of olive, and of this, 2.0 m³/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 m³/t of olive.²⁹ Greek style processing methods are less water-intensive using 0.9–1.9 m³/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 hydroxytyrosol⁴⁰ 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-

179 treated extracts, and control untreated extracts according to a
180 previously established method.⁵⁰

181 **Passive Adsorption of Phenolics to Resins.** A 5 g sample of
182 pretreated resin (i.e., FPX66, XAD4, XAD16N, or XAD7HP) was
183 mixed with 40 mL of olive extract and placed in a 250 mL Erlenmeyer
184 flask at 25 °C. The control contained 40 mL of olive extract and no
185 resin. Flasks were not sealed and were swirled by hand between
186 sampling. A 1 mL aliquot of supernatant was sampled after 4, 10, 16,
187 20, and 30 min. Replicate samples were taken at each time point and
188 the phenolic concentration quantified using UHPLC-ESI (MS/
189 MS).⁵⁰

190 **Resin-Assisted Olive Debittering.** Olives obtained from Musco
191 Olive Company were harvested on October 27, 2015, and shipped
192 that day at 25 °C from Tracy, California, to Davis, California. A
193 selection of raw olives was frozen on day 0 and stored in −80 °C until
194 sampled. Olives were treated with FPX66 resin by placing 15
195 unblemished green whole olives in 125 mL Erlenmeyer flasks with 25
196 g of activated FPX66 resin and 60 mL of 1.0% acetic acid in deionized
197 (DI) water (pH ~4). Controls were created by placing 15
198 unblemished green whole olives in 125 mL flasks with 60 mL of
199 1.0% acetic acid in DI water (pH ~4). Flasks were sealed until
200 sampling. Olives were sampled on days 0, 6, 26, 76, and 273. Olives,
201 brine, and resin were separated using a Buchner funnel and Whatman
202 No. 10 filter paper. Olives were separated into three composite
203 samples of 5 olives each, weighing approximately 26 g (wet weight).
204 Composite samples were blended (Waring WSG30 Commercial Spice
205 Grinder-120 V) and placed in a 50 mL conical tube. Lipids were
206 removed with three successive 10 mL aliquots of hexane. Tubes were
207 shaken vigorously for 1 min and centrifuged at 4000 rpm for 5 min.
208 The lipid layer was decanted and the defatted pulp frozen at −80 °C
209 for 12 h. Samples were then freeze-dried to a consistent weight, and
210 the resulting powder was sieved through a Tyler standard #65 screen
211 with a 0.210 mm opening. Compounds were extracted with a 1:40 w/
212 v of 60% methanol in water, with 1 min of agitation and centrifugation
213 at 4000 rpm for 5 min. Brine was sampled directly without extraction.
214 Olive extracts and brine were filtered through a 0.22 μm nylon filter
215 prior to UHPLC-ESI MS/MS analysis. Samples were diluted to be
216 within the linear dynamic range.

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

222 **Ultra-High-Performance Liquid Chromatography-Electron**
223 **Spray Ionization Tandem Mass Spectrometry (UHPLC-ESI)**
224 **MS/MS).** UHPLC analysis was performed according to the previously
225 described method.⁴⁴ Briefly, compounds were analyzed on an Agilent
226 1290 Infinity ultra-high-performance liquid chromatography system
227 (UHPLC) interfaced to a 6460 triple-quadrupole mass spectrometer
228 (MS/MS) with electrospray ionization (ESI) via Jet Stream
229 technology (Agilent Technologies, Santa Clara, CA, USA). The
230 UHPLC was equipped with a binary pump with an integrated vacuum
231 degasser (G4220A), an autosampler (G4226A) with thermostat
232 (G1330B), and a thermostated column compartment (G1316C).
233 Compounds were separated using a Poroshell 120 C₁₈ column (3.0 ×
234 50 mm, 2.7 μm, Agilent Technologies). The mobile phase consisted
235 of a linear gradient, flowing at 0.7 mL/min, of 0.01% acetic acid in
236 purified water (A) and 0.01% acetic acid in acetonitrile (B) as follows:
237 10% B, 0–2 min; 10–30% B, 2–3 min; 30–65% B, 3–5 min. The
238 column temperature was 20 °C, and the injection volume was 5 μL.
239 Oleuropein, oleuropein aglycone, ligstroside, ligstroside aglycone,
240 hydroxytyrosol, tyrosol, oleocanthal, and oleacein were quantified
241 against purified standards. Ligstroside was quantified using relative
242 quantification against oleuropein standards.

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

RESULTS

247 Weather extremes are recognized to pose a significant 248
249 challenge to food systems in recent years and are likely to 249
250 become even more important in the future. In California, 250
251 drought, fire, and water use are primary concerns, creating a 251
252 new paradigm for food manufactures that rely heavily on 252
253 unrestricted water use for food processing. One such industry 253
254 is the table olive industry.

255 Herein, XAD4, FPX66, XAD16N, and XAD7HP (Table 1) 255 t
256 were evaluated for the ability to adsorb oleuropein, ligstroside, 256

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

^aInformation obtained from product specification sheets provided by Rohm and Hass.^{37–40}

257 oleuropein aglycone, ligstroside aglycone, oleocanthal, ole- 257
258 acein, hydroxytyrosol, and tyrosol from whole olives during 258
259 typical brine storage as a method for decreasing water use in 259
260 table olive processing.

261 Initially, olive extracts were treated with XAD4, FPX66, 261
262 XAD16N, and XAD7HP for 16 h to assess the ability of the 262
263 resins to bind the compounds in olives which are related to 263
264 olive bitterness. The phenolics were quantified in the olive 264
265 extracts and in materials recovered from resins using UHPLC- 265
266 (ESI) MS/MS. These results are reported as percentage 266
267 remaining after 16 h of resin exposure in Table 2. All resins 267 t
268 demonstrated the ability to reduce levels of oleuropein, 268
269 ligstroside, oleacein, and oleuropein aglycone below the limit 269
270 of detection at 16 h. Ligstroside aglycone and oleocanthal were 270
271 below the limit of detection in the initial extracts (i.e., time 0). 271
272 When compared to initial conditions, XAD4, FPX66, and 272
273 XAD16N significantly reduced levels of hydroxytyrosol to 273
274 15.1–25.7% and tyrosol to 12.2–16.9% of the initial 274
275 concentration in olive extracts, whereas reduction on the 275
276 XAD7HP resin was significantly lower for hydroxytyrosol (40.1 276
277 ± 7.3% remaining of initial concentration) and tyrosol (26.9 ± 277
278 4.3% remaining of initial concentration). XAD7HP is an 278
279 aliphatic resin with a smaller surface area (>380 m²g^{−1}) as 279
280 compared to the aromatic FPX66, XAD4, and XAD16N resins. 280
281 This may have contributed to the lower adsorption observed 281
282 on the XAD7HP resin (Table 1). Recoveries of phenolic 282
283 compounds from the resins were excellent for all phenolics of 283
284 interest: 66.5–73.1% oleuropein, 68.3–75.3% oleuropein 284
285 aglycone, 68.0–74.1% ligstroside, 43.0–64.8% oleacein, 285
286 89.4–102.5% tyrosol, and 80.2–89.4% hydroxytyrosol. 286

287 To evaluate the kinetics of phenolic adsorption to the resins, 287
288 olive extracts were exposed to resins and sampled at 4, 10, 16, 288
289 20, and 30 min. Levels of olive phenolics were quantified in 289
290 these samples (Figure 2a–e). Levels of oleuropein, ligstroside, 290 t

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%

^aExpressed as a percentage of original concentration in olive extract at time 0 min.

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.

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.

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,

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 ± 0.287

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 ($\alpha = 0.05$) 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 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

compound	day	control olives brine	resin-treated olives	
			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%

^aConcentrations are expressed as a percentage of original concentration in initial olives at time 0 min.

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, ligstroside, 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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