

# Influence of California-Style Black Ripe Olive Processing on the Formation of Acrylamide

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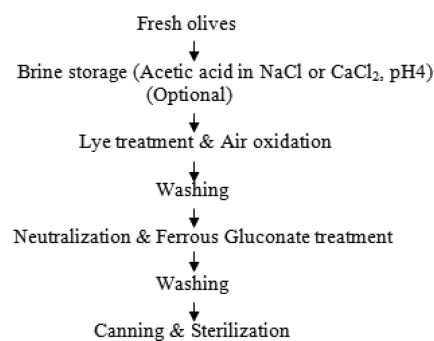
**ABSTRACT:** Methods used in processing California-style black ripe olives generate acrylamide. California-style black ripe olives contain higher levels of acrylamide ( $409.67 \pm 42.60$ – $511.91 \pm 34.08 \mu\text{g kg}^{-1}$ ) as compared to California-style green ripe olives ( $44.02 \pm 3.55$ – $105.79 \pm 22.01 \mu\text{g kg}^{-1}$ ), Greek olives ( $<1.42 \mu\text{g kg}^{-1}$ ), and Spanish olives (not detected), indicating that the higher temperatures used to sterilize the California-style green ripe and black ripe olives are required for acrylamide formation. Preprocessing brine storage influenced the formation of acrylamide in a time-dependent manner. Acrylamide increased during the first 30 days of storage. Longer brine storage times ( $>30$  days) result in lower acrylamide levels in the finished product. The presence of calcium ions in the preprocessing brining solution results in higher levels of acrylamide in finished products. Air oxidation during lye processing and the neutralization of olives prior to sterilization significantly increase the formation of acrylamide in the finished products. Conversely, lye-processing decreases the levels of acrylamide in the final product. These results indicate that specific steps in the California-style black ripe olive processing may be manipulated to mitigate the formation of acrylamide in finished products.

**KEYWORDS:** olive, *Olea europaea*, acrylamide, California-style black ripe olives

## INTRODUCTION

Olive fruit is derived from a small evergreen tree in the family Oleaceae, genus *Olea*. California is the only U.S. state to commercially produce olives. About 50% of the production is destined for canning as in California-style black olives; another 46% is pressed into olive oil.<sup>1</sup> Raw olive fruit is very bitter due to the presence of high concentrations of phenolic compounds and in particular the *o*-diphenol oleuropein. Before olives can be consumed, they must be cured (processed) to remove the bitter components. There are numerous methods used worldwide to cure olives. One of the most popular is the California-style processing method created by Freda Ehmann in the 1890s.<sup>2</sup> To make California-style black ripe olives, the olives are picked green (unripe) and are cured by exposing them to a series of lye and oxygenated water baths over multiple days.<sup>3</sup> The main olive variety grown in California and used in the production of California-style black ripe olives is Manzanilla.<sup>4–6</sup> In 2012, canning olive production was 78,500 tons, of which 86% was Manzanilla olives.<sup>6</sup> Olives are harvested in California between September and November. Olives that cannot be processed directly after harvesting are stored in acidic brine solution (0.2–0.4% calcium chloride) to prevent the growth of spoilage organisms until they can be processed. During processing, the fresh or brine-stored olives are immersed in a 1–2% sodium hydroxide solution (lye) for 2–24 h over 3–7 consecutive days. During the intervals between lye treatments, the olives are suspended in water and air is bubbled through the tank to oxidize the olives and form the black color (air oxidation). These pigments, formed through oxidation and polymerization of *o*-diphenols (primarily hydroxytyrosol and caffeic acid), are not stable.<sup>3</sup> To prevent color deterioration, iron salts such as ferrous gluconate, ferrous sulfate, or ferrous lactate are added to fix the color. Olives are then neutralized with lactic acid or carbon dioxide, packed into cans in a sodium

chloride solution, and sterilized. In the United States, olives are sterilized at 115.6 °C for 60 min or at 121.1 °C for 50 min.<sup>2,3</sup> A diagram of the steps involved in California-style black ripe olive processing is shown in Figure 1.



**Figure 1.** Processing diagram for the production of California-style black ripe olives.

Recently, the carcinogen acrylamide was found in California-style black ripe olives at relatively high concentrations ( $226$ – $1925 \mu\text{g kg}^{-1}$ ) as compared to other foods such as French fries ( $20$ – $1325 \mu\text{g kg}^{-1}$ ), baked goods ( $<364 \mu\text{g kg}^{-1}$ ), and nut products ( $<457 \mu\text{g kg}^{-1}$ ).<sup>7</sup> Acrylamide is classified as a probable human carcinogen (group 2A) by the International Agency for Research on Cancer on the basis of studies in laboratory animals.<sup>8</sup> In 2002, the Swedish National Food Administration and the University of Stockholm announced the finding of high

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levels ( $\mu\text{g kg}^{-1}$  to  $\text{mg kg}^{-1}$ ) of acrylamide in foods cooked at elevated temperatures.<sup>9</sup> This finding led to worldwide concern and increased research activities investigating the formation and mitigation of acrylamide in foods.<sup>9–14</sup> Although the relevance to human health of dietary exposure to acrylamide is unclear, regulatory agencies such as the World Health Organization (WHO) continue to encourage food manufacturers to take measures to reduce acrylamide levels in processed foods.<sup>15</sup>

In general, acrylamide is formed through reactions between free amino acids and carbonyl compounds (e.g., reducing sugars) via the Maillard reaction at temperatures above 120 °C.<sup>9–14</sup> Stable isotope-labeling studies in potato-based foods and model systems indicate that the backbone of acrylamide arises from asparagine.<sup>14</sup> Alternative formation mechanisms have also been suggested to occur in lipid-rich foods.<sup>16,17</sup> For example, studies of model systems containing asparagine and unsaturated lipids demonstrate that the aldehydes and ketones, produced through the thermal degradation of lipids, can react with asparagine and lead to acrylamide formation.<sup>16</sup> Additionally, acrylamide was found to form through the reaction of acrolein and/or acrylic acid with ammonia.<sup>16</sup> Acrylic acid is generated from the oxidation of acrolein, a product of thermal degradation and/or oxidation of free fatty acids or glycerol.<sup>18</sup> In these cases, the backbone of acrylamide would arise from either acrylic acid or acrolein, and the amine group derived from the ammonia evolved from the thermal degradation of amino acids.<sup>16</sup>

The formation of acrylamide during California-style black ripe olive processing was studied by Casado and Montañó in 2008.<sup>19</sup> These studies demonstrated that there were undetectable amounts of acrylamide in the olives before sterilization, whereas the sterilized olives contained acrylamide ranging from 243.30 to 1349.00  $\mu\text{g kg}^{-1}$ . This information suggests that acrylamide in California-style black ripe olives forms during sterilization. These studies also show very low concentrations (52.80–198.00  $\mu\text{g kg}^{-1}$ ) of asparagine in the nonsterilized olives and no correlation between the concentration of glucose and amino acids (both total and individual amino acids) and acrylamide formation.<sup>19</sup> More recently, using olive juice in a model system, Casado et al. proposed that peptides and proteins were responsible for acrylamide formation in sterilized olives and that the California-style black ripe olive processing method had no effect on acrylamide levels in the final products.<sup>20</sup>

To date, information on the mechanism of acrylamide formation in California-style black ripe olives is limited, and it remains unclear if acrylamide is formed through the California-style black ripe processing method or if it is an artifact of the sterilization process. Herein, we evaluate the effect of individual processing steps employed in the California-style black ripe olive processing method (i.e., brine storage, lye treatment, air oxidation, neutralization, and sterilization) on the formation of acrylamide to better understand the formation mechanism of acrylamide during processing and to provide information for mitigating acrylamide formation through processing.

## MATERIALS AND METHODS

**Reagents.** All reagents were of analytical grade unless otherwise stated. Acrylamide (99+%), D<sub>3</sub>-Labeled acrylamide (2,3,3-D<sub>3</sub>, 98.0%), and formic acid (~98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol and hexane were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

**Olives.** To estimate acrylamide levels in various table olives, olives including Greek-style, Spanish-style, California-style black ripe, and California-style green ripe olives were studied. Three different commercial brands of each table olive style were purchased from local markets in at least triplicate over two years (2010 and 2014). A 15 kg sample of fresh and a 70 kg sample of brine-stored olives were kindly supplied by two independent commercial olive processors located in central California (processor A and processor B). These olives were graded as medium-sized Manzanilla harvested in 2009 and 2012. Olives represent pooled samples from numerous orchards. Directly after receipt of fresh olives, the processors prepared brine-stored olives and delivered both fresh and brine-stored olives to the University of California, Davis. Brine-stored olives were stored on site in brine for 6 months at room temperature before they were analyzed for acrylamide.

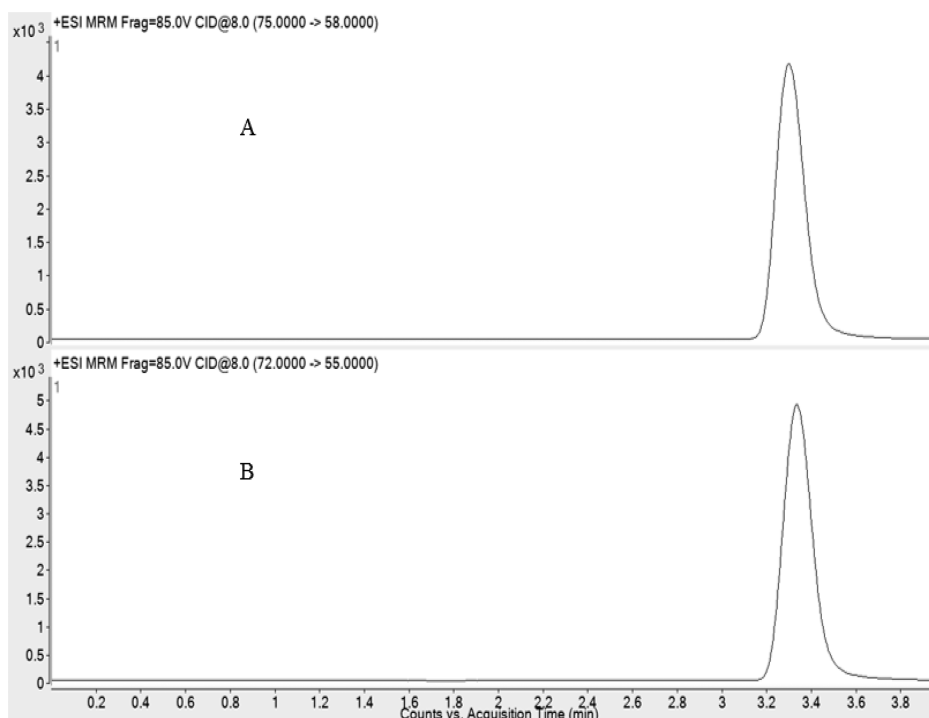
Graded medium-sized Manzanilla olives harvested in 2010 and 2011 were used to study the influence of the individual processing steps on acrylamide formation. A 30 kg sample of fresh and a 70 kg sample of brine-stored olives were also supplied by processors A and B and represent pooled samples from numerous orchards.

**Laboratory-Scale Models Mimicking California-Style Black Ripe Olive Processing.** A laboratory-scale model of the California-style black ripe olive processing method was developed for these studies. This model system enabled duplicate processing of small-scale samples (~30 olives per sample). Briefly, olives were hand selected for no defect and placed into one of two sample chambers. The olives were exposed to a 1% sodium hydroxide solution for 5 h for lye processing. Next, the olives were rinsed and placed in fresh water for 19 h to remove residual sodium hydroxide. During this washing step, air was bubbled into the surrounding medium to oxidize olives. This lye–wash cycle was repeated for 4 days to allow sodium hydroxide to penetrate to the pit of the olive. A solution of 1% phenolphthalein in isopropanol was used to verify the penetration depth of the sodium hydroxide. After the final lye–wash, olives were treated with 0.15% ferrous gluconate solution for 4 h to fix the color. During the ferrous gluconate treatment, carbon dioxide gas was bubbled into the solution to neutralize olives and phenolphthalein solution was used to verify the pH of olives. When the pH was <8, the olives were rinsed and put into fresh water for 30 min to remove residual ferrous gluconate. Olives were then packed in a glass flask, which was filled with 2% sodium chloride. Finally, the olives were sterilized in an autoclave at a temperature of 127 °C for 30 min.

**Brine Storage.** To evaluate the influence of brining on acrylamide levels in the finished olive product, olives (harvested in 2010 and 2011) were stored in a standardized brine solution for 0–8 months at room temperature prior to processing as described above. Experiments were performed in duplicate. Preprocessing brine solutions reflected typical commercial brine solutions and were composed of calcium chloride (0.2–0.4%) and sodium benzoate (0.2–0.3%). The pH of the brine solutions was maintained at 3.7–4.0 with acetic acid. The influence of calcium chloride, sodium benzoate, or acetic acid was evaluated in olives harvested in 2011 from the two olive processors. This was achieved by omitting one of these individual components in three independent brine solutions and storing the olives in these solutions at room temperature for 3 months prior to processing as described above. The study was performed in duplicate.

**Effect of Lye, Air Oxidation, Ferrous Gluconate, Neutralization, and Sterilization.** Olives (harvested in 2010 from the two olive processors) were processed as described above. Individual processing parameters (i.e., air oxidation step, ferrous gluconate treatment, neutralization, and sterilization) were systematically omitted, and the levels of acrylamide were evaluated in the resulting products. The olives were stored in brine for 6 months prior to these studies. The studies were performed in duplicate.

**Analysis of Acrylamide.** *Preparation of Samples for Acrylamide Analysis.* Samples consisting of 30 g of olives were extracted with 60 mL of ultrapure water. A 1 mL aliquot of 4 ng mL<sup>-1</sup> of D<sub>3</sub>-acrylamide was added to samples as an internal standard. The mixture was homogenized. Twenty-five grams of the mixture was collected, and 15 mL of hexane was added to remove oil and other nonpolar



**Figure 2.** UHPLC-(ESI) MS/MS ion select chromatogram of D<sub>3</sub>-labeled acrylamide (a) and acrylamide (b).

compounds. Then the mixture was centrifuged at 4000 rpm for 30 min. The aqueous layer was collected. Interfering compounds were removed, and sample was concentrated using solid phase extraction on a Strata-X-C cartridge (3 mL, 200 mg) preconditioned with two 1 mL aliquots of methanol, followed by two 1 mL aliquots of water at a flow rate of 2 mL min<sup>-1</sup>. Acrylamide was eluted with 1 mL of 0.1% formic acid in water/methanol, 90:10, v/v. The eluted solution was collected and filtered through a 0.22 μm membrane prior to analysis by ultrahigh-pressure liquid chromatography electrospray ionization mass spectroscopy [UHPLC-(ESI)MS/MS].

**Acrylamide Analysis by UHPLC-(ESI)MS/MS.** Acrylamide was quantified in samples using a modified method of Zhang et al.<sup>21</sup> Samples were analyzed using an Agilent 1290 Infinity ultrahigh-pressure liquid chromatography (UHPLC) system coupled to an Agilent 6460 triple-quadrupole mass spectrometer interfaced with electrospray ionization (ESI) via jet stream technology (Agilent Technologies, Santa Clara, CA, USA). The UHPLC was equipped with a binary pump with integrated vacuum degasser (G4220A), an autosampler (G4226A), and a thermostated column compartment (G1316C). The olive extracts were separated on a Polaris C18-A column (2.0 × 150 mm, 3 μm, Agilent Technologies), and the column temperature was controlled at 30 °C. The mobile phase consisted of 0.1% formic acid in water (A) and methanol (B) as follows: 0% B, 0–1 min; 0–20% B, 1–2 min; 20% B, 2–4 min, at a flow rate of 0.2 mL/min. The column was re-equilibrated between injections for 4.5 min with 0.1% formic acid in water. The injection volume was 5 μL. To quantify acrylamide levels in olives, transition ions of  $m/z$  72 → 55 for acrylamide and  $m/z$  75 → 58 for D<sub>3</sub>-labeled acrylamide were monitored. The UHPLC-(ESI)MS/MS was optimized using a fragmentor voltage of 85 V, a collision energy of 8 V, and a cell accelerator of 7 V in positive mode ESI. The drying gas temperature and flow rate were 300 °C and 5 L/min, respectively. The sheath gas temperature and flow rate were 400 °C and 11 L/min, respectively. The nebulizer gas pressure, nozzle voltage, and capillary voltage were 45 psi, 500, and 3000 V, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) of this method were 0.71 and 1.42 μg kg<sup>-1</sup>, respectively.

**Measurement of pH of Olives.** To measure the pH of olives, five olive fruits were homogenized and the pulp was filtered through

cheesecloth. The pH was measured in the resulting olive juice using a SevenMulti pH meter (Mettler Toledo, Columbus, OH, USA).

**Statistical Analysis.** Means (duplicates or triplicates) of acrylamide concentrations in olives were statistically analyzed using one-way ANOVA followed by multiple-comparison test using Tukey (HSD). The difference among samples was at the 95% confidence level. ANOVA was performed using XLSTAT version 2013.

## RESULTS AND DISCUSSION

The acrylamide concentrations of fresh, brine-stored, Greek-style, Spanish-style, California-style black ripe, and California-style green ripe olives were measured using UHPLC-(ESI)MS/MS monitoring transition ions of  $m/z$  72 → 55 for acrylamide and  $m/z$  75 → 58 for D<sub>3</sub>-labeled acrylamide (Figure 2). The results are presented in Table 1. Acrylamide was undetected in fresh and brine-stored olives. Acrylamide levels in processed olives, over two harvest years, were not significantly different. Significant amounts of acrylamide were present in the California-style black ripe (409.67 ± 42.60–511.91 ± 34.08 μg kg<sup>-1</sup>) and California-style green ripe olives (44.02 ± 3.55–105.79 ± 22.01 μg kg<sup>-1</sup>) but not in the Greek-style or Spanish style olives. California-style green ripe olives are processed fresh (not brine stored) and are not subject to air oxidation or ferrous gluconate treatment. California-style black ripe and green ripe processing methods required the olives to be sterilized at temperatures >110 °C.<sup>2,3</sup> In contrast, Spanish- and Greek-style table olive processing methods preserve olives using additives and/or pasteurization at temperatures <65 °C.<sup>2</sup> These results are in agreement with those obtained from Casado and Montaña and indicate that sterilization plays an important role in the formation of acrylamide.<sup>19</sup> The difference in the levels of acrylamide found in the California-style black ripe and green ripe olives indicates that other processing steps (i.e., brine storage, air oxidation, and ferrous gluconate treatment) also influence the levels of acrylamide in the final



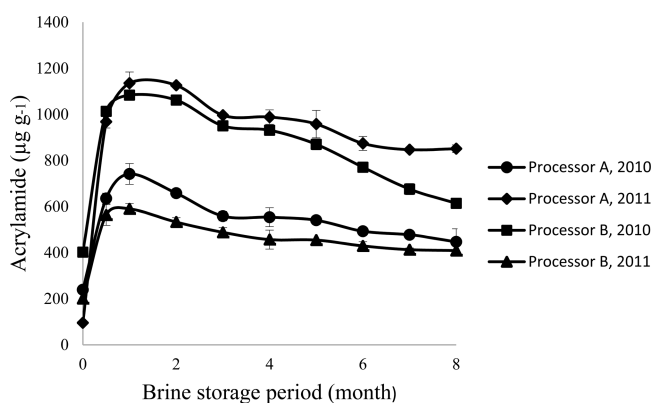
**Table 1. Acrylamide Levels in Fresh, Brine-Stored, Spanish-Style, Greek-Style, California-Style Black Ripe and California-Style Green Ripe Olives<sup>a</sup>**

olive sample	acrylamide in olive ( $\mu\text{g kg}^{-1}$ )	
	year 1	year 2
fresh	<0.71	<0.71
brine-stored	<1.42	<0.71
Spanish-style, brand A	<0.71	<0.71
Spanish-style, brand B	<0.71	<0.71
Spanish-style, brand C	<0.71	<0.71
Greek-style, brand D	<0.71	<0.71
Greek-style, brand E	<1.42	<0.71
Greek-style, brand F	<0.71	<0.71
California-style black ripe, brand G	493.45ab $\pm$ 48.99	511.91a $\pm$ 34.08
California-style black ripe, brand H	419.61c $\pm$ 48.28	409.67c $\pm$ 42.60
California-style black ripe, brand I	499.84ab $\pm$ 12.07	458.66bc $\pm$ 29.82
California-style green ripe, brand G	44.02e $\pm$ 3.55	46.15 e $\pm$ 7.81
California-style green ripe, brand H	84.49de $\pm$ 10.65	105.79d $\pm$ 22.01
California-style green ripe, brand J	50.41e $\pm$ 6.39	54.67e $\pm$ 7.81

<sup>a</sup>Results are mean values of three measurements. Means with different letters are significantly different at the 95% confidence level. There were 10 different commercial brands (A–J) used in the survey of acrylamide levels in olives. Fresh and brine-stored olives were received in 2009 and 2012. Commercial table olives (Greek-style, Spanish-style, California-style green, and California-style black ripe olives were purchased in 2010 and 2014.

product. Therefore, each of these parameters was evaluated independently as discussed below.

The influence of brining olives was evaluated by monitoring acrylamide in olives stored in brine over 8 months and processed into California-style black ripe olives. Acrylamide levels were lowest in processed fresh olives ( $95.85 \pm 8.52$ – $402.57 \pm 12.07 \mu\text{g kg}^{-1}$ ) as compared to processed brine-stored olives ( $408.96 \pm 2.84$ – $1135.29 \pm 48.99 \mu\text{g kg}^{-1}$ ). During storage, the acrylamide levels increased during the first month and then declined with increasing storage time (Figure 3). Acrylamide levels were highest in olives stored in brine for 1 month ( $590.01 \pm 23.43$ – $1135.29 \pm 48.99 \mu\text{g kg}^{-1}$ ). These data indicate that precursors of acrylamide formation either degrade

**Figure 3.** Effect of brine storage time (0–8 months) on acrylamide formation.

or diffuse into the surrounding medium during prolonged brine storage. This trend was observed in olives received from both olive processors and over the two harvest years (2010 and 2011). This result suggests that holding olives in brine may facilitate the reduction of acrylamide in final products.

The influence of the composition of preprocessing brine solutions was evaluated to identify relationships between individual brining components (i.e., calcium chloride, sodium benzoate, or acetic acid) and the formation of acrylamide (Table 2). The complete brine solution was composed of

**Table 2. Effect of Brine Composition on Acrylamide Formation in California-Style Black Ripe Olives<sup>a</sup>**

brine composition	acrylamide ( $\mu\text{g kg}^{-1}$ )
sample a (complete brine)	685.86 $\pm$ 26.98a
sample b (brine without sodium benzoate)	642.55 $\pm$ 44.02a
sample c (brine without acetic acid)	563.74 $\pm$ 36.21a
sample d (brine without calcium chloride)	303.17 $\pm$ 43.31b

<sup>a</sup>Means with different letters are significantly different at the 95% confidence level.

calcium chloride (0.2–0.4%) and sodium benzoate (0.2–0.3%) and acidified to a pH of 3.7–4.0 with acetic acid (sample a). The olives stored in brine solutions without added acidic acid (sample b) or sodium benzoate (sample c) did not differ significantly from the brine solution containing these compounds (sample a). However, the olives stored in the brine solution without added calcium chloride (sample d) had significantly lower levels of acrylamide in the finished products ( $303.17 \pm 43.31 \mu\text{g kg}^{-1}$ ). In general, calcium ions help maintain structural firmness, stability of cell wall, and cell turgor of fruits by forming cross-linkages between pectin molecules, thereby strengthening and preventing the collapse of plant cells.<sup>22,23</sup> Our data suggest that calcium in the brine solutions helps retain precursors to acrylamide formation by improving cell wall integrity and that acrylamide levels in finished products may be reduced through the omission of calcium in brining solutions. The olives stored in the brine solution with no calcium chloride addition appeared to shrink to a greater degree than olives stored with calcium chloride during sterilization. Although it appears that acrylamide could be reduced through lowering the calcium content of the storage brine, consumer acceptance may be affected.

The influence of the individual processing steps involved in the production of California-style black ripe olives on acrylamide formation was evaluated and is given in Table 3. The levels of acrylamide in fully processed California-style black

**Table 3. Effect of Air Oxidation, Ferrous Gluconate Treatment, and Sterilization on Acrylamide Levels in Olives<sup>a</sup>**

processing method	acrylamide ( $\mu\text{g kg}^{-1}$ )
complete (lye, air oxidation, ferrous gluconate, sterilization)	569.42a $\pm$ 38.34
no air oxidation (lye, ferrous gluconate, sterilization)	310.27b $\pm$ 0.71
no ferrous gluconate (lye, air oxidation, sterilization)	545.99a $\pm$ 28.40
no air oxidation or ferrous treatment (lye, sterilization)	344.35b $\pm$ 14.91
no sterilization (lye, air oxidation, ferrous gluconate)	51.83c $\pm$ 12.78

<sup>a</sup>Means with different letters are significantly different at the 95% confidence level.

ripe olives (i.e., the olives that were subjected to lye hydrolysis, air oxidation, ferrous gluconate treatment, and sterilization) were the highest ( $569.42 \pm 38.34 \mu\text{g kg}^{-1}$ ). In comparison, olives processed without the air oxidation step had significantly lower levels of acrylamide ( $310.27 \pm 0.71 \mu\text{g kg}^{-1}$ ). Ferrous gluconate had no effect on acrylamide levels. Olives processed with no air oxidation or ferrous gluconate treatment ( $344.35 \pm 14.91 \mu\text{g kg}^{-1}$ ) were not significantly different from olives processed with no air oxidation (Table 3). Air oxidation significantly increases the formation of acrylamide during processing. Comparisons of the sterilized olives with non-sterilized olives demonstrate low levels of acrylamide ( $51.83 \pm 12.781 \mu\text{g kg}^{-1}$ ) in nonsterilized olives as compared with sterilized olives and confirmed that acrylamide forms during sterilization.

Lye processing was found to significantly decrease the levels of acrylamide (and/or its precursors) in finished California-style black ripe olives. The levels of acrylamide in fresh ( $11288.29 \pm 5.68 \mu\text{g kg}^{-1}$ ) or brine-stored olives ( $7694.27 \pm 37.63 \mu\text{g kg}^{-1}$ ) that were directly sterilized were 10 times greater than levels found in olives that underwent lye processing for 4 days ( $569.42 \pm 38.34 \mu\text{g kg}^{-1}$ ) prior to sterilization. This finding is in contrast to the results reported by Casado et al.,<sup>20</sup> who suggested that lye treatment did not affect acrylamide formation in olives. However, this difference may be explained by the use of olive juice in the Casado et al. study and the use of olive fruit in the current study.

Neutralization with carbon dioxide after lye treatment had a significant influence on the formation of acrylamide in olives (Table 4). The initial pH of the lye-treated olives was 9.76;

**Table 4. Effect of Neutralization with Carbon Dioxide (pH) on Acrylamide Formation in California-Style Black Ripe Olives<sup>a</sup>**

neutralization time (h)	measured pH	acrylamide ( $\mu\text{g kg}^{-1}$ )
0	9.76	$338.67d \pm 9.23$
1	8.61	$437.36c \pm 4.97$
2	8.29	$585.04b \pm 0.71$
3	7.65	$591.43b \pm 19.88$
4	7.11	$681.60a \pm 11.36$

<sup>a</sup>Means with different letters are significantly different at the 95% confidence level.

after neutralization with carbon dioxide, the pH was reduced to 7.11. The lowest level of acrylamide ( $338.67 \pm 9.23 \mu\text{g kg}^{-1}$ ) was found in sterilized olives that were not previously neutralized with carbon dioxide, whereas the highest level of acrylamide ( $681.60 \pm 11.36 \mu\text{g kg}^{-1}$ ) was found in sterilized olives that had been neutralized. These results indicated that the formation of acrylamide in olives is pH dependent. This is in agreement with the results reported by Casado et al.<sup>24</sup>

The studies described herein demonstrate that the formation of acrylamide is dependent upon the processing methods used in the production of table olives and that sterilization temperatures are required for the formation of acrylamide. Additionally, our results indicate that fresh olives contain precursors of acrylamide which either degrade and/or diffuse into surrounding medium during brine storage. Calcium ions in brine storage solutions appear to prevent the short-term diffusion of acrylamide and/or its precursor from olives into brine storage solutions. Longer term (>1 month) brine storage results in lower acrylamide levels in finished products. The

observation that the air oxidation step results in increased levels of acrylamide in olives suggests that acrylamide precursors form via oxidation reactions during lye treatment and that these are converted into acrylamide during sterilization. Neutralization prior to sterilization increases acrylamide formation during sterilization, indicating that this is a pH-dependent process. These results suggest that employing small modifications during traditional processing methods (e.g., holding olives for longer periods in brining solutions, decreasing oxygen exposure time, reducing sterilization temperatures, etc.) may be useful in lowering acrylamide levels in finished table olive products.

Ongoing studies in our laboratory are focused on identifying the precursors of acrylamide in olives and determining the mechanism by which they form in olives.

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### Notes

The authors declare no competing financial interest.

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