Evaluation of Thermal Processing Variables for Reducing Acrylamide in Canned
Black Ripe Olives

**Shuaikun Tang^a, Roberto J. Avena-Bustillos^{b*}, Molly Lear^a, Ivana Sedej^c, Dirk M.
Holstege^d, Mendel Friedman^b, Tara H. McHugh^b, and Selina C. Wang^{a,c*}**

^a *Department of Food Science and Technology, University of California, Davis, One Shields Ave., Davis, CA 95616, USA*

^b *Healthy Processed Foods Research, Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan St., Albany, CA 94710, USA*

^c *Olive Center, University of California, Davis, One Shields Ave., Davis, CA 95616, USA*

^d *University of California Analytical Laboratory, University of California, Davis, One Shields Ave., Davis, CA 95616, USA*

* Corresponding author.

E-mail address: scwang@ucdavis.edu (Selina C. Wang)

Running title: Acrylamide Mitigation in Olive Processing

ABSTRACT

Acrylamide, formed in certain plant foods at elevated cooking temperatures, has been identified as a probable carcinogen. A wide variation in acrylamide concentration has been reported for commercial canned black ripe olives, with some levels being quite high. Standards for olive sterilization were developed before acrylamide was discovered to be present in food. The objective of this study was to determine if alternative sterilization conditions during thermal processing could substantially reduce acrylamide levels in black ripe olives, while still maintaining safety and quality. Heat penetration tests for six thermal processes were used to evaluate process F_0 – a sterilization value –which was correlated to acrylamide formation and changes in quality attributes of black ripe olives. Acrylamide concentration followed a positively correlated second order polynomial regression with process F_0 . This correlation was further demonstrated by two of the experimental processes with different initial temperatures and different processing times, but with ultimately similar F_0 , that produced very similar levels of acrylamide. The amounts of solids leaching from olives in brine increased at higher temperatures of thermal processing, whereas pH variation in olives and brine was most likely related to the differences in lye-wash cycles. Skin color was unaffected, whereas increasing (time/temperature) thermal processing reduced the firmness of whole olives. Optimization of safe thermal processing conditions while lowering process F_0 is a practical and efficient strategy to reduce acrylamide formation and improve the safety of black ripe olives.

Keywords. acrylamide reduction, black ripe olives, thermal processing, LACF lethality.

Chemical compounds used in this study:

Acrylamide (PubChem CID: 6579)

1. Introduction

Acrylamide [$[\text{CH}_2=\text{CH}-\text{CO}-\text{NH}_2]$] is a conjugated reactive molecule that acts as a biological alkylating agent. It has been reported to induce numerous adverse effects in cells, animals, and possibly also humans, such as antifertility, carcinogenicity, neurotoxicity, and teratogenicity (Friedman, 2003). Acrylamide has been identified as a probable carcinogen (International Agency of Research on Cancer - IARC, 1994; National Toxicology Program, 2012). The chemistry of acrylamide formation in food, and its *in vivo* reactions with essential enzymes, DNA, and neurons that cause the mentioned adverse effects as well as methods to reduce formation in processed food and mitigation of *in vivo* toxicity are described in detail elsewhere (Friedman, 2003; Friedman, 2015; Friedman and Levin, 2008; Friedman and Mottram, 2005).

Dietary acrylamide in most foods is derived from the heat-induced Maillard-type reaction between the amino group of the amino acid asparagine and the carbonyl group of a reducing sugar such as glucose and fructose. Both precursors and heat must be present for this route of formation, so acrylamide is generally found only in thermally processed plant-derived foods including cereals, coffees, almonds, olives, and potatoes (Friedman, 2003; Stadler et al., 2004; Tareke et al., 2002; Zyzak et al., 2003). Many reported methods to reduce acrylamide levels in foods depend on reducing the availability of the precursors, asparagine and sugars (Amrein et al., 2007; Friedman and Levin, 2008). The formation of acrylamide in olives is also heat-dependent, but appears to be by a different mechanism than the Maillard reaction (Casado et al., 2013; Charoenprasert and Mitchell, 2014). There is no correlation between asparagine and sugar concentrations in the raw olive, and subsequent acrylamide formation (Casado and Montaña, 2008). Acrylamide in olives is primarily found in the style known as California Black Olives,

which are sterilized at high temperature, rather than pasteurized or preserved as are most other styles (Charoenprasert and Mitchell, 2014).

Acrylamide is formed in foods cooked at elevated and typical baking and frying temperatures. Studies with laboratory-heated foods revealed a temperature dependence of acrylamide formation, which is not detected in unheated or boiled foods (Tareke et al., 2002). Various levels of acrylamide have been found in black ripe olives. In a survey of black ripe olives in US market, a wide range of acrylamide (375-1925 $\mu\text{g/kg}$) was found (U.S. Food and Drug Administration - U.S. Department of Health and Human Services - Center for Food Safety and Nutrition, 2006). Casado and Montaña (2008) screened 11 commercial black ripe olive samples (pitted, whole, and different cultivars) from Spain and found levels of acrylamide also ranged broadly from 176 to 1578 $\mu\text{g/kg}$ of olive pulp. Similarly, we found 288-1192 $\mu\text{g/kg}$ acrylamide in seven black ripe olives samples (unpublished results). Charoenprasert and Mitchell (2014) also reported relatively high concentrations of acrylamide in black ripe olives (226-1925 $\mu\text{g/kg}$).

It has been shown that acrylamide in black ripe olives is primarily formed during the sterilization step in the canning and bottling process, and that sterilization time and temperature can significantly influence the formation of acrylamide (Amrein et al., 2007; Casado and Montaña, 2008; Casado et al., 2010). Black ripe olives are processed through several lye immersion and water rinsing steps (Charoenprasert and Mitchell, 2014; Romero et al., 1995) and then canned or bottled and sterilized as a low-acid canned food (LACF) with a flesh pH around neutrality (Casado et al., 2007; Romero et al., 1995). LACF heat treatment standards require assurance of at least 12 log reduction of *Clostridium botulinum* spores at a 12D processing time at a constant lethal temperature, where D refers to time at a reference constant lethal temperature

to achieve a one log cycle reduction (or 90% reduction) in the number of spores in the LACF (Stumbo, 1973).

California-style black ripe olives, as a LACF, usually require sterilization temperatures above 110°C (Charoenprasert and Mitchell, 2014). In contrast Spanish- and Greek-style table olives are considered acidified foods (AF) and require less rigorous methods: either pasteurization temperatures below 65°C, additives for preservation, or a combination of the two, and as a consequence, acrylamide content is negligible in these two types of olives (Charoenprasert and Mitchell, 2014). Although acrylamide formation has been reported as temperature dependent, it correlates better with the overall thermal load, which also considers processing time. Lowering the sterilization temperature and shortening the sterilization time could reduce the formation of acrylamide, while continuing to meet the necessary standards to assure a low hazard and quality product.. It is hypothesized that the wide variation of acrylamide in commercial canned black ripe olives is a consequence of different thermal processes used by industry due to variable container sizes and retort types. Although it has been reported that acrylamide in canned black ripe olives is caused by high temperature processing (Casado and Montaña, 2008; Casado et al., 2010), there is a lack of systematic studies to define the effect of thermal processing during commercial sterilization of olives on the levels of acrylamide in the final product. For LACF, it is important to assure commercial sterility and then determine if it is still possible to reduce acrylamide concentrations below levels found while using commercial canning practices.

The objective of this study was to determine if different safe sterilization conditions during thermal processing could reduce substantially the acrylamide levels in canned black ripe olives. We adjusted the sterilization time and temperature for six different processing conditions. From

heat penetration data, process lethality was calculated and correlated to acrylamide formation and to changes in physical quality attributes of black ripe olives.

2. Materials and methods

2.1. Reagents

For the olive preparation, sodium hydroxide solution (50%) was obtained from Loeffler Chemical Corp. (Atlanta, GA, USA). Sodium chloride (canning and pickling salt, food grade) was purchased from Morton Salt Inc. (Chicago, IL, USA). Ferrous gluconate (food grade) was purchased from VWR (Radnor, PA, USA).

For the acrylamide analysis, acrylamide (>99.9%) and methanol (HPLC grade, 99.9%) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Acrylamide-d₃ standard solution (500 mg/L in acetonitrile) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Reversed-phase solid phase extraction cartridge columns (Sep-PakTM C18, 1 g and 6 cc) were obtained from Waters Corp. (Milford, MA, USA).

2.2. California-style black ripe olive preparation

Pilot-plant olive preparation was modified from the methods developed by Charoenprasert and Mitchell (2014) to mimic industrial California-style black ripe olive processing.

2.2.1. Harvest and washing

Olives were harvested in October, 2014 and stored in 2% acetic acid solution by the processor. During May 2015 13 kg batches of olives were immersed in 1% sodium hydroxide solution (19 L) for 5 h for lye treatment, and then rinsed eight times before immersion in fresh water for 19 h to remove the residual sodium hydroxide. During the immersion steps, air was

131 constantly bubbled into the water to oxidize the olives. The lye-wash cycle was performed a
132 second time with the following changes: during the second cycle, the olives were soaked in lye
133 for 2 h and immersed in fresh water for 20 h. The pH was measured after the final lye treatment
134 to ensure the lye penetrated to the pit and the pH was between 8 and 9.5. Then the olives were
135 treated with 0.15% ferrous gluconate solution (19 L) for approximately 3 h to fix the color.
136 During the ferrous gluconate treatment, carbon dioxide was bubbled into the solution to
137 neutralize olives. When the pH reached 7, the olives were rinsed to remove residual ferrous
138 gluconate.

139 2.2.2. *Blanching, canning and heat penetration testing*

140 Cruess (1921) indicated that a tendency of the can to "buckle" was greatly reduced when
141 olives in cans were thoroughly heated in an exhaust box to 90.6°C before sealing. To achieve
142 this, whole olives should be blanched under saturated steam conditions for 2-4 min. This
143 additional processing step eliminates tissue gases and bulging of cans without increasing
144 acrylamide levels. Whole olives were blanched for a residence time of 4 min., in single layers on
145 stainless steel trays moving on a continuous belt on a custom built steam blancher at the
146 University of California, Davis FST food processing pilot plant. Whole olives reached a
147 maximum temperature of $96.7 \pm 1.8^{\circ}\text{C}$ from an initial temperature of $25.0 \pm 1.1^{\circ}\text{C}$ ($n = 6$).

148 Can filling was done by hand. Fill weights (790 g) for 401×411 (10.32 cm diameter, and
149 10.91 cm height) cans were 322.5 g (41%) whole olives and 467.5 g (59%) previously boiled
150 brine (2-2.5% NaCl in water). A ¼ inch headspace in cans facilitated internal agitation during
151 intermittent rotation of cans inside the rotary retort.

152 To record the temperature of the can contents during processing and after sealing, digital
153 thermometers, Model 7500T DataTrace MPIII wireless data loggers (Mesa Laboratories, Inc.,

Lakewood, CO, USA), were installed in each of six cans before sealing using S.S. bendable retainers to fix the 1" (2.54 cm) rigid probe tip at the geometric center of cans. Sealing was done with a Dixie Double Seamer model UVGMD-ALCC (Dixie Canner Co., Athens, GA, USA) using the vacuum setting indicated for 401×411 cans. After sealing, cans were immediately sterilized.

To sterilize, a steam-heated discontinuous rotary retort (FMC Rotary Steritort, Madera, CA, USA), built in 1963 and refurbished in 2010 by Precision Canning Equipment Co. (Woodland, CA, USA), was used. Saturated steam sterilizes the product in sealed cans. Intermittent agitation induced convection heating every time the cans rotated freely in the bottom of the internal retort body. This rotary retort requires 10-17 min to reach 101.7°C for venting, and another 2-8 min for coming-up-time (CUT), depending on processing temperatures. This retort can run up to 72 cans spaced in a weight-balanced fashion. Retort was adjusted to constant processing temperatures in the range of 110.0°C-126.7°C for different experimental processing times. After cooling, cans with data loggers were opened and data collected with a laptop computer through a DT Pro MPIII PC Interface with DT Pro Basic software.

2.2.3. *Assessment of commercial sterilization of canned black ripe olives*

The heat penetration data obtained with the aid of the wireless data loggers (above) was used to establish lethality values to assure inactivation of *Clostridium botulinum* spores and reduction of other more thermo-resistant spoilage bacterial spores. Process lethality was calculated as process F_0 according to the evaluation of the integral alternative using the following equation that defines required or process F_0 lethality, either from microbiological or heat penetration data (Stumbo, 1973):

$$F_{T_{ref}}^Z = D_{T_{ref}}(\log(C_a) - \log(C_b)) = \int_{t_a}^{t_b} 10^{\frac{T(t) - T_{ref}}{Z}} dt \quad (1)$$

In this equation, in order to calculate F_0 as an integral equation with the DT Pro Basic software and Excel spreadsheet, $Z = 10^\circ\text{C}$ and $T_{ref} = 121.1^\circ\text{C}$, where $T(t)$ was temperature inside the cans during all the heating and cooling sterilization cycle from the start of the thermal processing (t_a) to the end of cooling (t_b), taken at 15 sec intervals (dt).

In the design of this study, safe F_0 thermal processing times for different retort temperatures and intermittent convection heating modes were applied according to process recommendations defined by Stumbo et al. (1975) and the state of California Department of Health Services (1995) in Table 1.

For LACF, thermal processing must assure very low probabilities of survival of thermophilic spoilage bacterial spores and even lower probabilities of survival of *Clostridium botulinum* spores (Stumbo et al., 1975). Sterilizing value F_0 is defined as the time in minutes at 250°F equivalent to destroy bacterial spores with a thermal sensitivity of $Z = 10^\circ\text{C}$ (indicating that there is a ten-fold increase in the rate of spore destruction when temperature is raised 10°C). Lethality of these spores is defined as the ratio of process F_0 to required F_0 and is measured by: a) microbial survival or b) the sum of different lethality as the temperature rises and decreases during the heating and cooling process as indicated by Equation 1. Required F_0 is determined not only by microbiological control needs, but previous industry process results, unique processing equipment, or even subjective concerns on process safety factors (Pflug, 2005). Process F_0 represent actual conditions in laboratory tests or the canning industry and could become required F_0 . The goal of commercial sterilization is to achieve lethality equal to one (unit lethality), i.e. when process F_0 is equal to required F_0 , as lethality is defined as the ratio of process F_0 /required F_0 .

199 2.3. Acrylamide analysis

200 2.3.1. Sample preparation

201 Whole canned black ripe olives (~20 g) were drained and crushed in a mortar with a pestle.
202 Sample pulp (2 g) was placed in a centrifuge tube and spiked with 0.5 µg d₃-acrylamide as an
203 internal standard. Water (4 mL) was added to the centrifuge tube. After 10 min shaking, hexane
204 (1 mL) was added, followed by further shaking for 10 min. The samples were then centrifuged at
205 8,000 rpm in a Sorvall SS-34 centrifuge (Thermo Scientific, Waltham, MA) for 10 min to
206 separate the aqueous and hexane layers. The aqueous layer was vacuum filtered using a 125 mL
207 Buchner funnel. Nitrogen was then blown on the top of the filtrate to remove residual hexane.

208 A Sep-Pak C18 cartridge (Waters, Milford, MA) was activated with methanol (2 mL)
209 followed by water (2 mL). The filtrate was then loaded on the cartridge and passed through
210 without vacuum (about 1.5 mL/min). The eluate was collected and evaporated to a volume of
211 less than 1 mL followed by addition of water to a volume of exactly 1 mL. Acrylamide
212 determination was performed by LC-MS/MS.

213 2.3.2. LC-MS/MS

214 The quantification of acrylamide/d₃-acrylamide was performed on a Sciex API 2000 triple-
215 quadruple MS system (Perkin-Elmer, Shelton, CT, USA) controlled by Analyst 1.4.2 software
216 (Applied Biosystems, Foster City, CA, USA). HPLC instrumentation consisted of a Perkin-
217 Elmer Series 200LC system with a quaternary pump, autosampler, and in-line mobile phase
218 degasser. The samples were separated using a Hypersil-Keystone Hypercarb column (50 ×
219 2.1mm, i.d., particle size 5µ; Thermo, Waltham, MA, USA). The mobile phase was isocratic
220 methanol/water (80:20, v/v) at 200µL/min for a total run time of 5 min. The column was

operated at room temperature. Injection volume was 35 μ L. The retention time of acrylamide and d_3 -acrylamide was 1.56 min.

The mass spectrum data were acquired with positive ion atmospheric pressure ionization (APCI) utilizing the multiple-reaction monitoring (MRM) mode. The ion source settings were as follows: temperature was 350°C; the curtain gas, collision gas, gas 1 and gas 2, 50, 6, 40 and 10 psi, respectively; the nebulizer current, 6 kV. The declustering potential, focusing potential, entrance potential, collision energy and collision cell exit potential were 55, 400, 1.6, 17 and 7.2 V, respectively. Transitions for acrylamide and d_3 -acrylamide were monitored at m/z (mass-to-charge ratio) $72 \rightarrow m/z 55$ and $m/z 75 \rightarrow m/z 58$, respectively with dwell times of 250 μ sec.

2.3.3. *Quantitation*

Quantitation was performed by internal calibration using the ratio of integrated area of acrylamide against d_3 -acrylamide. The calibration solutions were injected before, in the middle of, and after the analysis of the samples. The calibration curve used weighted (1/x) second-order regression. The calibration range is 19.70 – 5,000 ng/ml with the correlation coefficient (R^2) 0.996.

2.3.4. *Method validation*

The method was validated by determining the accuracy and precision of authentic acrylamide added (spiked) at 100 and 500 μ g/kg ($n=2$). All the results are the averages of duplicates. Method precision was assessed by measuring the repeatability of the results of one sample ($n=5$).

2.4. *Physicochemical properties of black ripe olives and brine*

Three cans from each thermal treatment were used for physicochemical analyses of ripe black olives and brine. All analyses were done in five replicates per can. After opening a can, olives were drained for 5 min and then both olives and brine were weighed.

2.4.1. Soluble solids and pH

Approximately half of the olives were depitted, blended in a laboratory blender (Waring Commercial, Torrington, CT, USA) and squeezed through a cheese cloth to obtain liquid for soluble solids and pH determination. Soluble solids were determined with a digital refractometer, LR-01 (Maselli Misure S.p.A., Parma, Italy). Sample pH was measured using a Beckman Φ 40 Series pH meter (Beckman Coulter, Fullerton, CA, USA).

2.4.1. Color of skins

Tristimulus (L^* , a^* , b^*) color values of whole olive skins were measured using a portable spectrophotometer model CM-508c (Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA) through a 3 mm diameter circular opening.

2.4.2. Texture

Texture analysis was performed on the TA-TX2 texture analyzer (Stable Micro Systems, Ltd, Godalming, Surrey, UK) with a puncture stainless steel probe (3 mm diameter, 25 mm long). Texture analyzer settings were as follows: operation mode – measure force in compression, pre-test speed – 10.0 mm/s, test speed – 1.0 mm/s, post-test speed – 10.0 mm/s, distance – 2.0 mm, trigger type – auto, trigger force – 0.20 N. Olives were cut with a razor blade alongside without touching the pit to obtain a flat surface. The probe reached olives' skin on the opposite side from the flat surface. The described setup allowed testing the firmness of the olives. The recorded parameters were maximum force area (g.s), maximum force (g), and time (s). Temperature of

olives immediately after texture analyses was measured with an Omega HH176 data logger thermometer with a precision fine wire thermocouple (Omega Engineering, Inc., Stamford, CT, USA).

2.4.3. Statistical analysis

Data were statistically processed by One-Way ANOVA using Minitab® Release 14.12.0 statistical software (Minitab Inc., State College, MA, USA) program. Means were compared by HSD Tukey's family error test at the significance level of $p \leq 0.05$. Treatment replicates varied from 2 to 15 depending on analysis accuracy and sample property variability.

3. Results and discussion

3.1. Thermal processing evaluation of canned black ripe olives

Table 1 reports required F_0 from four processing standards for different retort temperatures that can be used for the design of thermal processing of canned black ripe olives, as a LACF. For the best quality, the optimum value is the lowest F_0 required for safety assurance. The olive canning industry can use these required F_0 values to estimate process F_0 for different can sizes and retort systems. As there are several combinations of processing time and temperature used to obtain a process F_0 equal to the required F_0 for unit lethality, this allows industry to use alternative time/temperature profiles to affect a change in the acrylamide levels in the final product, i.e. processes which employ the highest retort temperatures with the minimum amount of time that can practically be achievable for their retort systems and can sizes.

Table 2 reports the sterilization conditions for the six different thermal processes applied in this study. Initial temperature was obtained from the wireless data loggers at the start of the

thermal processes. The average temperature of the brine in filled 401×411 cans, just before vacuum sealing, was $37.6 \pm 5.7^{\circ}\text{C}$. This value is within range of initial values during commercial runs, 21.1 to 43.3°C , as reported by the California Department of Health Services (1995). Venting time and coming-up time varied depending on steam feeding rate and retort initial temperatures. Processing time, which was calculated at different retort processing temperatures to achieve a target process F_0 (equation 1), was determined from preliminary heat penetration data obtained from wireless thermocouples inside 401×411 cans filled with whole black ripe olives and brine. The target F_0 is in alignment with process F_0 commonly used by industry and in our food pilot plant batch discontinuous rotary retort.

3.2. Effect of thermal processing on acrylamide formation in canned black ripe olives

Table 2 reports the acrylamide concentrations measured in six different thermal processes. Reported processing temperatures and corresponding times were chosen to obtain given F_0 processes. Initial temperatures, venting and coming-up times depended on processing equipment conditions, Acrylamide values reported in Table 2 are within the ranges found in screening studies of commercial black ripe olives from the USA and Spain (Casado and Montaña, 2008; U.S. Food and Drug Administration - U.S. Department of Health and Human Services - Center for Food Safety and Nutrition, 2006). Acrylamide concentration increased significantly as process F_0 reached up to 2.7 times the required F_0 values necessary to assure a high and safe degree of commercial sterility for the can size used in this study (Stumbo et al., 1975). Statistical analysis on treatments $110^{\circ}\text{C}/175$ min and $115.6^{\circ}\text{C}/45$ min showed very similar process F_0 (11.26-11.91), and no significant differences in acrylamide concentrations (154.2 – 156.2 $\mu\text{g}/\text{kg}$), respectively. These observations support our theory that acrylamide formation in olives is associated with total heat treatment (time and temperature) in the form of process F_0 , and not the

individual parameters. However, Montañó et al. (2008) reported at conference that for similar thermal processes, or analogous cumulative lethality expressed by process F_0 , acrylamide formation was lower for the processes carried out at higher temperature. Because their study was not published, we were unable to compare our results to the actual process F_0 values or temperature/time processing conditions.

From the results of this study, it appears that the main reason there are wide-ranging reports of acrylamide levels in canned black ripe olives may be due to a range of different process F_0 used by the olive canning industry. The California Department of Health Services (1995) advises to use a required F_0 of 13 min as an official required sterilization control process for olives, ripe, whole, pitted or broken pitted, in brine in 300×407 (7.62 cm diameter and 11.27 cm height) cans.

Mathematical fitting of the relationship between process F_0 and acrylamide concentration reported in Table 2 follows a second order polynomial equation ($\text{Acrylamide, } \mu\text{g/kg} = 1.64F_0^2 - 2.08F_0$) with high correlation coefficient ($R^2 = 0.9932$). This non-linear regression as shown in Figure 1, valid only for the range of F_0 tested, is typical of biological and chemical phenomena. However, a slight extrapolation of this polynomial equation to process F_0 values of 32-34 min, usually obtained in olives canned at the highest retort temperatures (123.9-129.4 °C), gives acrylamide concentrations values in the range of 1750-1950 $\mu\text{g/kg}$, as found in samples of commercial canned black ripe olives in USA and Spain.

According to our polynomial equation, a required F_0 of 13.0, as prescribed by the State of California (Table 1), would lead to an acrylamide concentration in canned black ripe olives of 250 $\mu\text{g/kg}$. This concentration is within the range of the lowest acrylamide concentrations found in commercial canned black ripe olives by Charoenprasert and Mitchell (2014) and by our group (unpublished results) of 226 and 288 $\mu\text{g/kg}$, respectively. It is important to consider that an

acrylamide concentration of 250 µg/kg, as would be formed by adhering to the current required F_0 established by the California Department of Health Services (1995), is not a serious health hazard, considering the low consumption of canned black ripe olives compared with (a) fried potato products and chips; b) crackers, cookies and cakes; and (c) bread - the most important food sources of acrylamide in the USA diet at 38%, 17%, and 14% of total dietary acrylamide, respectively (Friedman and Levin, 2008).

Moreover, on the basis of the lower required F_0 for LACF reported by Stumbo et al. (1975), the olive canning industry could petition for the reduction of the current required thermal processing standard to a new F_0 of 10 min (300×407 cans under convection heating), thus obtaining commercial sterility with a high degree of assurance, without jeopardizing health and safety. This could potentially reduce acrylamide concentration to 150 µg/kg; a very significant reduction. In preliminary studies, Casado and Montaña (2008) varied sterilization time from 15 to 35 min at 121°C and suggested that by optimizing sterilization conditions it could be possible to reduce the acrylamide concentration to <100 µg/kg in Hojiblanca variety ripe olives. However, the influence of temperature of sterilization on acrylamide formation were not covered in their study. We suggest that the canning industry invest in devising new optimized thermal processes suited to specific industrial conditions, either by hiring an external Thermal Processing Authority or using their in-house Thermal Processing Authority teams.

Alternative approaches to reduce acrylamide in black ripe olives by making changes in the traditional processing steps were explored by Charoenprasert and Mitchell (2014) and include: a) brining for an extended time of 2-8 months before sterilization; b) removing calcium chloride from the brine solution; c) decreasing air oxidation during lye treatment; and d) decreasing carbon dioxide neutralization after lye treatment. These four modifications achieved reductions

in acrylamide concentrations of 25, 55.8, 45.5, and 14.2%, respectively from control concentrations (Table 3). However, the reported effects on sensory properties of the three last options could apparently negatively affect the quality of the final product. For this study we employed the first strategy of extending the brining time to seven months, which was the time period reported by Charoenprasert and Mitchell (2014) to result in the lowest acrylamide formation. Irrespective of this fact, acrylamide increased to the highest possible values at the highest process F_0 values applied in this study, similar to the highest acrylamide concentrations reported by Charoenprasert and Mitchell (2014) if processed for one month of brining, suggesting the importance of reducing the thermal processing conditions while optimizing thermal processing.

Casado et al. (2010) evaluated the effects of different additives (salts, amino acids and antioxidants) to reduce acrylamide in canned black ripe olives. Most of these additives were unsuccessful, however, 25 mM sodium bisulfite reduced acrylamide completely without any negative effect on sensory quality (Table 3). Unfortunately, sodium bisulfite is allergenic and requires a labeling warning by food safety regulations. In another study, López-López et al. (2014) concluded that after sulfite, the compounds that had the highest impact on acrylamide reduction in thermal processed ripe olives (up to 22% of control values) were proline and sarcosine (Table 3). Sánchez et al. (2014) tested the sensory characteristics of olives treated with these amino acids and found panelist could usually identify the presence of the additive, with sarcosine being more acceptable than proline. None of the strategies reported in Table 3 could reduce acrylamide to the levels achievable by optimized thermal processing, as demonstrated in this study, without certain negative effects on quality, production costs, or regulatory constraints.

3.3. Effect of thermal processing on physical attributes of brine and whole black ripe olives

Use of a lower process F_0 was not associated with any loss of quality. Table 4 shows that the soluble solids in the brine increased only at the highest process F_0 (27.7). This increase can be explained by the leaching of pulp as the whole olive starts to break down at the higher temperature. The pH of the brine and whole olives varied, most likely as a result of different batch lye-wash cycles. Although Casado et al. (2007) reported a pH decrease due to the harsh thermal processing conditions of black ripe olives and hypothesized it could be related to organic acid formation from sugars degradation and/or degradation of Maillard reaction products or saponification of pectin ester groups, our results show that olives processed with the highest F_0 had the highest pH relative to the olives processed under the milder conditions.

Table 5 lists some quality aspects for olives processed under different F_0 . In opposition to the findings of Casado et al. (2007), who reported loss of green color and increased darkening by increasing thermal processing, presumably due to Maillard reactions and autoxidation of phenolic compounds, the color parameters in this study were not significantly different. The firmness of whole olives was significantly reduced with increasing thermal processing. Romero et al. (1995) also reported a decrease in firmness of canned black ripe olives at increasing process F_0 , indicating that changes in firmness of ripe olives during sterilization follows first order kinetics, with activation energy values within the range reported for fruits and vegetables.

4. Conclusions

The optimization of thermal processing conditions is a practical and efficient strategy to reduce acrylamide formation to improve the safety of canned black ripe olives. A modification of thermal processing conditions will require the olive canning industry to file new processes with the Food and Drug Administration (FDA). The proposed processes should be supported by

specific heat penetration studies and thermal processing evaluation conducted by LACF thermal processing authorities.

Acknowledgment

We appreciate the support of Bell-Carter Foods, Inc. for providing brined whole Manzanilla olives for this study and Jane Yegge for technical information about industry practices for processing black ripe olives. We also appreciate the support of Mr. Mark Deniston, Associate Director, Process Engineering at IEH Incorporated for providing information on thermal processing recommendations for whole black ripe olive canning. ST and SCW thank Dajun Yu, Yuyi Cao and Karinna Estay for assistance with olive preparation. The authors appreciated the valuable manuscript editing support provided by Carol E. Levin from the Healthy Processed Foods Research unit at Western Regional Research Laboratory, Agricultural Research Service, U.S Department of Agriculture.

Funding Sources

Funding was provided by the California Olive Commission.

References

- Amrein, T.M., Andres, L., Escher, F., Amadò, R. (2007). Occurrence of acrylamide in selected foods and mitigation options. *Food Additives & Contaminants*, 24(Suppl 1), 13-25, <http://dx.doi.org/10.1080/02652030701242558>.
- California Department of Health Services (1995). State of California official state sterilization control process for olives, ripe, whole, pitted or broken pitted, in brine (300×407 cans) Sacramento, CA,
- Casado, F.J., Montañño, A. (2008). Influence of processing conditions on acrylamide content in black ripe olives. *Journal of Agricultural and Food Chemistry*, 56(6), 2021-2027, <http://dx.doi.org/10.1021/jf072960b>.
- Casado, F.J., Montañño, A., Spitzner, D., Carle, R. (2013). Investigations into acrylamide precursors in sterilized table olives: evidence of a peptic fraction being responsible for acrylamide formation. *Food Chemistry*, 141(2), 1158-1165, <http://dx.doi.org/10.1016/j.foodchem.2013.04.040>.
- Casado, F.J., Sánchez, A.H., Montañño, A. (2010). Reduction of acrylamide content of ripe olives by selected additives. *Food Chemistry*, 119(1), 161-166, <http://dx.doi.org/10.1016/j.foodchem.2009.06.009>.

- Casado, F.J., Sánchez, A.H., Rejano, L., Montañó, A. (2007). Study of new procedures of elaboration of alkali-treated green table olives, not fermented, preserved by heat treatments. *Grasas y Aceites*, 58(3), 275-282, <http://dx.doi.org/10.3989/gya.2007.v58.i3.183>.
- Charoenprasert, S., Mitchell, A. (2014). Influence of California-style black ripe olive processing on the formation of acrylamide. *Journal of Agricultural and Food Chemistry*, 62(34), 8716-8721, <http://dx.doi.org/10.1021/jf5022829>.
- Cruess, W.V. (1921). Some factors affecting the quality of ripe olives sterilized at high temperature, *University of California Publications, Bulletin No. 333*. College of Agriculture: Agricultural Experiment Station, Berkeley, California,
- Friedman, M. (2003). Chemistry, biochemistry, and safety of acrylamide. A review. *Journal of Agricultural and Food Chemistry*, 51(16), 4504-4526, <http://dx.doi.org/10.1021/jf030204+>.
- Friedman, M. (2015). Acrylamide: inhibition of formation in processed food and mitigation of toxicity in cells, animals, and humans. *Food & Function*, 6, 1752 - 1772, <http://dx.doi.org/10.1039/c5fo00320b>.
- Friedman, M., Levin, C.E. (2008). Review of methods for the reduction of dietary content and toxicity of acrylamide. *Journal of Agricultural and Food Chemistry*, 56(15), 6113-6140, <http://dx.doi.org/10.1021/jf0730486>.
- Friedman, M., Mottram, D. (2005). *Chemistry and safety of acrylamide in food*. Springer, New York, <http://dx.doi.org/10.1007/b106417>.
- International Agency of Research on Cancer - IARC (1994). Some Industrial Chemicals, *Monographs on the Evaluation of Carcinogen Risk to Humans*. International Agency for Research on Cancer, WHO, Lyon, France, p. 389, <http://monographs.iarc.fr/ENG/Monographs/vol60/mono60-16.pdf>.
- López-López, A., Beato, V.M., Sánchez, A.H., García-García, P., Montañó, A. (2014). Effects of selected amino acids and water-soluble vitamins on acrylamide formation in a ripe olive model system. *Journal of Food Engineering*, 120(0), 9-16, <http://dx.doi.org/10.1016/j.jfoodeng.2013.07.019>.
- Montañó, A., Casado, F.J., Rejano, L., Sánchez, A.H. (2008). Formación de acrilamida durante el tratamiento de esterilización de aceitunas tratadas con alcali, *Cytalia XIII: Congreso anual de Ciencia y Tecnología de Alimentos*, Madrid, pp. 9-11,
- National Toxicology Program (2012). Toxicology and carcinogenesis studies of acrylamide in F344/N rats and B6C3F1 mice. U.S. Department of Health and Human Services, http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr575_508.pdf.
- Pflug, I. (2005). Microbiological input to the calculation of thermal processes for low-acid canned foods (LACF), *Institute of Food Thermal Processing Specialists Annual Meeting*, San Antonio, TX, USA,
- Romero, C., García, P., Brenes, M., Garrido, A. (1995). Colour and texture changes during sterilization of packed ripe olives. *International Journal of Food Science & Technology*, 30(1), 31-36, <http://dx.doi.org/10.1111/j.1365-2621.1995.tb01942.x>.
- Sánchez, A.H., Beato, V.M., López-López, A., Montañó, A. (2014). Comparative study of the use of sarcosine, proline and glycine as acrylamide inhibitors in ripe olive processing. *Food Additives & Contaminants, Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment*, 31(2), 242-249, <http://dx.doi.org/10.1080/19440049.2013.871756>.
- Stadler, R.H., Robert, F., Riediker, S., Varga, N., Davidek, T., Devaud, S., Goldmann, T., Hau, J., Blank, I. (2004). In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. *Journal of Agricultural and Food Chemistry*, 52(17), 5550-5558, <http://dx.doi.org/10.1021/jf0495486>.
- Stumbo, C.R. (1973). *Thermobacteriology in Food Processing* (2nd ed). Academic Press, New York,
- Stumbo, C.R., Purohit, K.S., Ramakrishnan, T.V. (1975). Thermal process lethality guide for low-acid foods in metal containers. *Journal of Food Science*, 40(6), 1316-1323, <http://dx.doi.org/10.1111/j.1365-2621.1975.tb01080.x>.

- 476 Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Törnqvist, M. (2002). Analysis of acrylamide, a
477 carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry*, 50(17), 4998-
478 5006, <http://dx.doi.org/10.1021/jf020302f>.
- 479 U.S. Food and Drug Administration - U.S. Department of Health and Human Services - Center for Food
480 Safety and Nutrition (2006). Survey Data on Acrylamide in Food: Individual Food Products,
481 [http://www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm053549.ht](http://www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm053549.htm)
482 [m](http://www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm053549.htm).
- 483 Zyzak, D.V., Sanders, R.A., Stojanovic, M., Tallmadge, D.H., Eberhart, B.L., Ewald, D.K., Gruber, D.C.,
484 Morsch, T.R., Strothers, M.A., Rizzi, G.P., Villagran, M.D. (2003). Acrylamide formation mechanism
485 in heated foods. *Journal of Agricultural and Food Chemistry*, 51(16), 4782-4787,
486 <http://dx.doi.org/10.1021/jf034180i>.
487

488 .Figure 1. Effect of thermal process lethality Fo on acrylamide concentration in black ripe olives.

489

490

Tables

Table 1

Required F_0 (in minutes) for black ripe table olives as a low-acid canned food in 401×411 (10.32 cm diameter, and 10.91 cm height) can size.

Sterilization condition/Retort temperature	110°C	115.6°C	121.1°C	126.7°C
Assure high degree of safety ¹	6.38	4.68	4.17	3.58
Commercial sterility with moderate degree of assurance ¹	6.93	6.93	6.93	6.93
Commercial sterility with high degree of assurance ¹	10.40	10.40	10.40	10.40
Dept. of Health Services, State of California ²	—	13.0	13.0	13.0

¹Stumbo et al. (1975).

²California Department of Health Services (1995).

500

501 **Table 2**

502 Thermal processing variables and acrylamide concentration in black ripe olives processed in 401×411 (10.32 cm
 503 diameter and 10.91 cm height) cans in a discontinuous rotary retort.

Initial temperature (°C)	Venting time to 101.7°C (min)	Coming-up time (CUT) (min)	Processing temperature (°C)	Processing time (min)	F ₀ process (min)	Acrylamide (µg/kg)
33.3±0.6 ^c	10	12	110.0	90	6.32±0.02 ^a	63.7±15.9 ^a
33.3±0.2 ^c	11	13	115.6	45	11.26±0.04 ^b	154.2±27.9 ^b
41.4±1.7 ^d	11	13	110.0	175	11.91±0.19 ^c	156.2±25.0 ^b
23.7±0.2 ^a	17	24	121.1	10	13.15±0.24 ^d	284.9±49.8 ^c
27.2±0.9 ^b	14	21	121.1	15	17.97±0.19 ^e	539.7±38.5 ^d
44.1±1.4 ^e	14	22	126.7	5	27.66±0.43 ^f	1184.5±82.6 ^e

504 Initial temperature and F₀ process are means of five determinations ± standard deviation. Acrylamide
 505 concentrations are means of six determinations ± standard deviation. Different subscript letters indicate significant
 506 difference at p<0.05 by Tukey's difference tests.

507

508

509

510 **Table 3**

511 Effect of processing conditions and additives to reduce acrylamide formation in black ripe table olives.

Processing method	Acrylamide (µg/kg)	Acrylamide % reduction ⁽¹⁾	Comments	Reference
Reduced and neutralized with carbon dioxide, (pH 8.3 vs. 7.1)	585.0±0.7	14.2	Taste could be affected	Charoenprasert and Mitchell (2014) ⁽²⁾
Holding olives for more than one month in brine	450±50	25.0	Acrylamide content varied different processing times	Charoenprasert and Mitchell (2014) ⁽²⁾
No air oxidation	310.3±0.7	45.5	Color could be affected (less blackness)	Charoenprasert and Mitchell (2014) ⁽²⁾
Cysteine (50 mM) in fill brine	200	50	Taste could be affected	Casado et al. (2010) ⁽³⁾
Brine without calcium chloride	303.2±43.3	55.8	Olives might shrink during sterilization	Charoenprasert and Mitchell (2014) ⁽²⁾
Proline or sarcosine (100 mM) in fill brine	80	75	Sweet, unpleasant, strange flavor ⁽⁴⁾	López-López et al. (2014) ⁽³⁾
Sodium bisulfite (25 mM) in fill brine	Not detected	100	Allergenic sulfites are not allowed in table olives	Casado et al. (2010) ⁽³⁾

512 (1) Control acrylamide concentration varies according to specific batch processing conditions.

513 (2) Sterilization done at 127°C for 30 min.

514 (3) Sterilization done at 121°C for 30 min.

515 (4) Sánchez et al. (2014)

516

517 **Table 4**

518 Soluble solids and pH of black ripe olives and brines with different thermal treatments.

Process F ₀ (min)	Soluble solids (°Brix)		pH	
	Brine	Olives	Brine	Olives
6.32±0.02 ^a	2.72±0.11 ^a	3.99±0.09 ^{NS}	6.67±0.05 ^c	7.47±0.08 ^b
11.26±0.04 ^b	2.64±0.17 ^a	3.68±0.69	6.70±0.03 ^c	7.39±0.06 ^a
11.91±0.19 ^c	2.79±0.10 ^{ab}	3.70±0.09	6.55±0.06 ^{ab}	7.36±0.07 ^a
13.15±0.24 ^d	2.83±0.14 ^{ab}	3.48±0.11	6.53±0.04 ^a	7.40±0.02 ^a
17.97±0.19 ^e	2.62±0.06 ^a	3.33±0.08	6.59±0.03 ^b	7.38±0.06 ^a
27.66±0.43 ^f	2.97±0.21 ^b	3.51±0.36	6.67±0.06 ^c	7.64±0.13 ^c

519

520 Values are means of fifteen determinations ± standard deviation. Different subscript letters indicate significant

521 difference at p<0.05 by Tukey's difference tests.

522 ^{NS} No significant difference in olive pulp °Brix by different thermal treatments.

523
524
525
526
527
528
529
530

Table 5
Color and firmness properties of whole black ripe olives with different thermal processes.

Process F ₀ (min)	Color			Firmness	
	L*	a*	b*	Maximum force area (g.s)	Maximum force (g)
6.32±0.02 ^a	26.6±0.27 ^{NS}	0.19±0.12 ^{NS}	0.29±0.07 ^{NS}	170.1±11.1 ^b	141.8±11.6 ^{ab}
11.26±0.04 ^b	26.7 ±0.01	0.27±0.09	0.50±0.10	175.5±11.4 ^b	146.6±3.8 ^b
11.91±0.19 ^c	26.8±0.41	0.16±0.10	0.25± 0.18	160.6±4.9 ^{ab}	135.2±9.0 ^{ab}
13.15±0.24 ^d	26.9±0.28	0.30±0.06	0.33± 0.05	151.6±6.2 ^a	131.6±3.4 ^{ab}
17.97±0.19 ^e	26.7±0.43	0.14±0.06	0.30±0.08	149.8±8.2 ^a	135.9±8.0 ^{ab}
27.66±0.43 ^f	26.4±0.32	0.22±0.08	0.25±0.12	140.6±17.0 ^a	121.5±16.9 ^a

Values of color and firmness are means of fifteen determinations ± standard deviation. Different subscript letters in a column indicate significant difference at p<0.05 by Tukey’s difference tests.

^{NS} No significant difference in color parameters on olive skins by different thermal processes