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Cholesterol oxidation and astaxanthin degradation in shrimp during sun drying and storage

Josafat A. Hernández Becerra [a,b], Angélica A. Ochoa Flores [a,c], Gerardo Valerio-Alfaro [a], Ida Soto-Rodriguez [d], María T. Rodríguez-Estrada [e], Hugo S. García [a,*]

[a] UNIDA, Instituto Tecnológico de Veracruz, M.A. de Quevedo # 2779. Col. Formando Hogar. Veracruz, Veracruz 91897, Mexico
[b] División de Tecnología de Alimentos, Universidad Tecnológica de Tabasco, Kilómetro 14.6 Carretera Villahermosa-Teapa. Villahermosa, Tabasco 86280, Mexico
[c] División Académica de Ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco, Km 25 Carretera Villahermosa-Teapa. Villahermosa, Tabasco, Mexico
[d] Facultad de Bioanálisis, Universidad Veracruzana. Carmen Serdán s/n, Col. Flores Magón, Veracruz, Veracruz 91700, Mexico
[e] Dipartimento di Scienze e Tecnologie Agro-Alimentari, Alma Mater Studiorum-Università di Bologna, Viale G. Fanin, 40, 40127 Bologna, Italy

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ABSTRACT

Dried salted shrimps are made from raw shrimps, which are cooked and dried under direct sunlight. The preparation and storage include treatments and conditions that can promote oxidative changes in different components. The aim of this study was to monitor the formation of major cholesterol oxidation products and the changes in the astaxanthin content and fatty acid profile in dried salted shrimp during cooking, sun drying and storage. During sun drying, most of the astaxanthin (75%) was degraded in cooked shrimp, while cholesterol oxidation products (COPs) showed a dramatic increase (8.6-fold), reaching a total concentration of 372.9 ± 16.3 μg/g of lipids. Further storage favoured both astaxanthin degradation (83%) and COPs formation (886.6 ± 97.9 μg/g of lipids after 90 days of storage). The high degradation of astaxanthin and the elevated formation of COPs during sun drying and storage indicate the necessity to re-evaluate the processing and storage conditions of salted dried shrimp.

1. Introduction

Shrimp is one of the main marine products with strongest worldwide trade interests. Shrimp production has significantly increased in recent years, and it now ranked as the fishery product with the greatest economic impact in the world. Fresh and frozen shrimp are the main presentations under which this crustacean is marketed. However, some products as dried salted shrimp are manufactured in several countries to be used in the preparation of traditional foods. Dried salted shrimp is prepared by boiling raw shrimp in brine, dried under direct sunlight for 3–5 days, and packaged in plastic bags for bulk storage (Tirawanichakul, Naphatthalung, & Tirawanichakul, 2008).This process brings about several changes in structural and biochemical components that affect the overall sensory profile of the product (Niamnuy, Devahastin, & Soponronnarit, 2007). One of the most noticeable changes during cooking is colour, which turns to red during heating. Astaxanthin is the main pigment of crustaceans, and it is usually present in free form, esterified or bound to some macromolecules, such as proteins (caroteneproteins). Cleavage of this complex results in a marked colour change, due to the release of the carotenoid (Naguib, 2000).

During sun drying, shrimp moisture content decreases slowly depending on the sunlight availability. Several techniques of shrimp drying have been used with various degrees of success. In this regard, drying conditions exert a significant influence on quality parameters, such as shrinkage, rehydration, texture and colour (Niamnuy et al., 2007; Tirawanichakul et al., 2008).

Components of the lipid fraction of shrimp gain relevance when their effects on human health are considered. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most abundant polyunsaturated fatty acids in shrimps (Sriket, Benjakul, Visessanguan, & Kijroongrojana, 2007), which are well known for their health benefits (Ruxton, Reed, Simpson, & Millington, 2004). Cholesterol is another important component present in the lipid fraction of shrimp, whose concentration is usually greater than 100 mg/100 g raw shrimp (Sriket et al., 2007). Although cholesterol plays important biological roles in

Abbreviations: COPs, Cholesterol oxidation products; 7α-HC, 7α-Hydroxycholesterol; 7α-HC, 7α-Hydroxycholesterol; α-CE, 5,6α-Epoxycholesterol; α-CE, 5,6α-Epoxycholesterol; 7-KC, 7-Ketocholesterol; 20-HC, 20-Hydroxycholesterol; 25-HC, 25-Hydroxycholesterol; CT, Cholestanetriol; 7α-OOH, 7α-Hydroperoxycholesterol; 7β-OOH, 7β-Hydroperoxycholesterol; 5α-OOH, 5α-Hydroperoxycholesterol; PUFA, Polyunsaturated fatty acids; MUFA, Monounsaturated fatty acids; SFA, Saturated fatty acids; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid.

* Corresponding author. Tel.: +52 229 9345701x116.
E-mail address: hsgarcia@itver.edu.mx (H.S. García).

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human metabolism, it is highly susceptible to oxidation, thus leading to the formation of cholesterol oxidation products (COPs) that have shown adverse effects on human health, such as cytotoxicity, mutagenicity and carcinogenicity (Otaegui-Arrazola, Menéndez-Carreño, Ansorena, & Astiasarán, 2010). Moreover, COPs are involved in the initiation and progression of several chronic diseases, such as atherosclerosis, neurodegenerative disorders, diabetes and kidney failure (Otaegui-Arrazola et al., 2010). COPs present in human plasma may actually derive from *in vivo* oxidation of endogenous cholesterol or from exogenous dietary sources (Otaegui-Arrazola et al., 2010). The extent of cholesterol oxidation in food is affected by the type of food (composition of the food matrix and the presence of antioxidants or pro-oxidants), its processing, handling and storage conditions such as temperature and time of treatment, and exposure to UV/VIS light (Ohshima, 2002).

Traditional processing of dried salted shrimp involves several treatments and conditions that favour both lipid and cholesterol oxidation, leading to the occurrence of different oxidation products. Recent studies have reported the presence of significant quantities of COPs in commercial samples of dried salted shrimp acquired in different cities of Mexico and Brazil (Sampaio, Bastos, Soares, Queiroz, & Torres, 2006; Soto-Rodríguez, Campillo-Velazquez, Ortega-Martinez, Rodriguez-Estrada, Lercker & Garcia, 2008). However, information regarding the formation of COPs in dried salted shrimp during processing and storage, as well as concomitant changes in astaxanthin content and fatty acid profile, is limited. The aim of this study was to analyze the formation of the main COPs and the associated changes in the astaxanthin content and fatty acid profile in dried salted shrimp during cooking, sun drying and storage.

2. Materials and methods

2.1. Materials

Fresh white shrimp (*Litopenaeus vannamei*) was obtained from a local seafood market in Veracruz (Mexico). The average weight of individual shrimps was 22.5 ± 3.0 g (39–51 shrimp/kg); 2.5 kg of raw shrimp were used in the study, which were split in three groups and each subjected to processing in triplicate. The shrimp was maintained at 2 °C in an ice chest filled with crushed ice until cooked (about 3 h).

2.2. Boiling in brine and sun drying

Raw whole shrimp was kept at room temperature for 10 min before cooking in brine (5% NaCl solution, w/v). Cooking was performed for 15 min in a 25 cm diameter stainless steel pot, with a shrimp to brine mass ratio of 1:2 on weight basis. These conditions are similar to those used in Mexico for the traditional production of dried salted shrimp (Soto-Rodríguez et al., 2008). After boiling, the shrimp were separated from the brine and allowed to reach room temperature.

Cooked shrimps were spread on a plastic mesh and exposed to direct sunlight for periods of 8 h per day during 4 days. During sun drying, each shrimp was flipped every 2 h, to ensure similar exposure to sunlight on both sides.

2.3. Storage

The dried salted shrimp were packed in transparent polypropylene boxes (13 × 15 × 8 cm), under normal atmosphere. Sealed boxes containing nine shrimp were kept for 90 days at room temperature.

2.4. Sampling

Samples were taken during processing and storage as follows: (1) before boiling in brine (whole raw shrimp), (2) after boiling in brine (boiled shrimp), (3) every 8 h during sun drying and (4) every 15 days during storage. Three samples consisting of three randomly selected shrimps were taken at each of the stages described.

2.5. Reagents and chemicals

Analytical grade chemicals were purchased from Teqsiquim (Mexico City. Mexico). Standard of astaxanthin was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). COPs standards supplied by Sigma–Aldrich (Mexico City, Mexico) were: lup-20(29)-ene-3β,28-diol (betulin) used as internal standard for COPs quantication and 5α-cholestane used as standard for cholesterol quantification, cholest-5-en-3β-ol (cholesterol), cholest-5-en-3β-ol-7-one (7-ketocholesterol), 5α,6α-epoxy-cholestane-3β-ol (5,6α-epoxycholesterol), 5β,6β-epoxy-cholestane-3β-ol (5,6β-epoxycholesterol), cholestane-3β,5α,6β-triol (cholestanetriol), cholest-5-en-3β,7β-diol (7β-hydroxycholesterol), cholest-5-en-3β,20α-diol (20α-hydroxycholesterol) and cholest-5-en-3β,25-diol (25-hydroxycholesterol). The standard cholest-5-en-3β,7β-diol (7α-hydroxycholesterol) was supplied by Steraloids (Newport, CT, USA).

Solid-phase extraction (SPE) cartridges (500 mg aminopropyl stationary phase/3 mL) were purchased from Alltech (Mountain View, CA, USA). The silylation agent was a mixture of dried pyridine, hexamethyldisilazane and trimethylchlorosilane (all Sigma products) in a ratio of 5:2:1 (v/v/v).

Methanolic HCl (3 M) was purchased from Sigma–Aldrich (Mexico City, Mexico). The standard mixture of fatty acid methyl esters (Supelco 37 component FAME mix) was supplied by Sigma–Aldrich (Mexico City, Mexico).

2.6. Methods

2.6.1. Moisture analysis

Moisture content was determined by the AOAC (2000) method 950.46.

2.6.2. Sodium chloride analysis

Sodium chloride concentration was determined by the AOAC (2000) method 935.47.

2.6.3. Lipid extraction

Lipid extraction was performed according to Boselli, Velazco, Caboni and Lercker (2001). About 16 g (d.b.) of sample (three shrimps) were minced with a blade-type mixer, weighed exactly in a 500 mL screw-cap glass bottle, added with 200 mL of a mixture chloroform/methanol (1:1, v/v) and homogenized for 3 min with an Ultra-Turrax IKA® T25 Digital (Ika-Werke, Staufen, Germany). The bottle was placed in a water bath at 60 °C for 20 min before adding 100 mL of chloroform. After a 3-min homogenization, the entire content was filtered through Whatman No. 1 filter paper. The filtrate was mixed thoroughly with 100 mL of a 1 M KCl solution and left overnight at 4 °C to generate phase separation. The lower phase was collected and dried in a vacuum evaporator. Fat content was determined gravimetrically. The lipid extract was stored at −20 °C in *n*-hexane/isopropanol (3:2, v/v) until analysed within a period that did not exceed 48 h.

2.6.4. Fatty acids analysis by gas chromatography

Fatty acid methyl esters (FAME) were prepared according to Soto-Rodríguez et al. (2008). About 50 mg of each lipid extract

were mixed with 1 mL of 0.2 M methanolic HCl. This mixture was heated to 60 °C for 4 h and then 200 μL of distilled water were added. The resulting mixture was extracted with 2 mL of *n*-hexane, dried with anhydrous sodium sulfate and centrifuged at 4000*g* for 2 min. One microlitre of the methylated preparation was injected into a HP6890 gas chromatograph (Hewlett–Packard, Wilmington, DE, USA), equipped with a HP-INNOWax capillary column (polyethylene glycol) (60 m × 0.25 mm i.d. ×0.25 μm film thickness (Hewlett–Packard, Palo Alto, CA, USA). Oven temperature was programmed from 170 to 210 °C at a rate of 4 °C/min, held at 210 °C for 5 min, increased to 230 °C at a rate of 4 °C/min, and finally held at 230 °C for 20 min. Nitrogen was used as carrier gas at a flow rate of 2.0 mL/min. The injector and FID temperatures were both set at 250 °C. FAME peak identification was carried out by comparing the peak retention times with those of the Supelco 37 component FAME mix (Sigma–Aldrich, Mexico City, Mexico). FAME data were expressed as area percentage with respect to the total FAME area.

2.6.5. Cold saponification of lipids and extraction of the unsaponifiable matter

A 250-mg lipid sub-fraction of the extract was added with 150 μL of a solution of betulin (1.0 mg/mL in *n*-hexane/isopropanol (3:2, v/v)) and 200 μL of a solution of 5α-cholestane (5.0 mg/mL in *n*-hexane), used as internal standards for the quantiication of COPs and cholesterol, respectively. The sample was then dried under nitrogen flow and added with 10 mL of 1 M KOH solution in methanol, wrapped with aluminium foil and shaken for 18 h in order to produce saponification (Soto-Rodríguez et al., 2008).For the extraction of the unsaponifiable matter, 10 mL of water and 10 mL of diethyl ether were added to the samples, which were vigorously shaken. The diethyl ether fraction was then separated; diethyl ether extraction was repeated twice. Ether extracts were pooled and washed with 5 mL of 0.5 M KOH and 5 mL of saturated NaCl. The extracts were dried over anhydrous sodium sulphate. The organic solvent was removed with a rotary evaporator at 40 °C; the unsaponifiable fraction was then transferred to a conical vial with diethyl ether and dried under nitrogen flow. The unsaponifiable extract was dissolved in 1 mL of *n*-hexane/isopropanol (3:2, v/v), from which 100 and 900 μL were used for the determination of cholesterol and COPs, respectively.

2.6.6. GC-FID analysis of total cholesterol

One-tenth of the unsaponifiable matter was dried under nitrogen flow and subjected to silylation by adding 0.1 mL of the derivatizing mixture (pyridine/hexamethyldisilazane/trimethylchlorosilane, 5:2:1, v/v/v) at 40 °C for 15 min; thereafter, it was dried under nitrogen flow and dissolved in 100 μL of *n*-hexane (Sweeley, Bentley, Makita, & Wells, 1963). One microlitre of the silylated solution was injected into an Agilent 7820 gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA), equipped with a split-splitless injector and flame ionization detector (FID). A HP-5 fused–silica capillary column (30 m × 0.32 mm i.d. ×0.25 μm film thickness) coated with 5%-phenyl-methylpolysiloxane (Hewlett–Packard, Palo Alto, USA) was used. Oven temperature was programmed from 230 to 270 °C at a rate of 2 °C/min, then increased to 300 °C at a rate of 1 °C/min and finally held at 300 °C for 10 min. Nitrogen was used as carrier gas at a flow rate of 1.5 mL/min. Injector and FID temperatures were both set at 325 °C.

Total cholesterol was quantified by the internal standard method, using 5α-cholestane as internal standard (IS). Peak identification of cholesterol was carried out by comparing the peak retention time with that of the cholesterol standard and by spiking the samples with a small amount of cholesterol standard. Quantification of cholesterol was performed by using relative response factors (Guardiola, Bou, Boatella, & Codony, 2004), which were calculated using standard solutions with known concentrations of cholesterol and 5α-cholestane.

2.6.7. Purification and GC-FID analysis of COPs

The remaining 9/10 of the unsaponifiable matter were taken to dryness, re-suspended in 300 μL of *n*-hexane/ethyl acetate (95:5, v/v) and purified by NH_2 SPE (Rose-Sallin, Huggett, Bosset, Tabacchi & Fray, 1995). The unsaponifiable extract was loaded into the SPE cartridge, which had been previously equilibrated with 3 mL of *n*-hexane. The cartridge was eluted with the following solvent sequence: 6 mL of *n*-hexane/ethyl acetate (95:5, v/v), 10 mL of *n*-hexane/ethyl acetate (90:10, v/v) and 10 mL of acetone. COPs eluted with the acetone fraction, which was collected, silylated (Sweeley et al., 1963), dried under nitrogen flow and dissolved again in 100 μL of n-hexane. One microliter of the silylated COPs was injected into the GC under the same conditions used for the determination of total cholesterol. Total COPs were quantified by the internal standard method, using betulin as internal standard. Peak identification of COPs was performed by comparing the peak retention times with those of true standards and by spiking the samples with a small amount of a COPs standard mixture. Quantification of COPs from CG data was performed by using relative response factors, which were calculated using standard solutions with known concentrations of COPs and betulin (Guardiola et al., 2004).

2.6.8. Kinetic modeling of COPs formation

The empirical approach of a logistic equation proposed by Ozilgen and Ozilgen, 1990 was used to model the formation of the main COPs during sun drying of cooked shrimp. The logistic equation was:

$$C = \frac{c_0 e^{kt}}{1 - \frac{C_0}{C_{max}}(1 - e^{kt})} \quad (1)$$

where C is the concentration of the cholesterol oxidation product, C_{max} is the maximum attainable concentration of cholesterol oxidation products, k (h^{-1}) is the rate constant and t is time (h).

The linearized form of equation (1) is:

$$Ln\left(\frac{C_{max}}{C_0 - 1}\right) = kt - ln\left(\frac{X}{1 - X}\right) \quad (2)$$

where $X = C/C_{max}$. This equation provides a straight line of $In(X/(1-X))$ *vs.* t with the slope k and intercept $-In(C_{max}/C_0-1)$.

2.6.9. Astaxanthin analysis

Astaxanthin content was determined according to the method of Tolasa, Cakli, and Ostermeyer, (2005). About 10 g of sample were extracted trice with 40 mL of 0.05% butylated hydroxytoluene (BHT) solution (in acetone) with an Ultra-Turrax IKA® T25 Digital homogenizer (Ika-Werke, Staufen, Germany) for 2 min. Samples were cooled during homogenization to avoid warming up. After extraction, samples were centrifuged (Eppendorf centrifuge 5804R, Hamburg, Germany) at 4000*g* at 4 °C for 5 min. To separate the water insoluble compounds, the acetone extracts of the samples were transferred to a 250 mL separating funnel with 40 mL of *n*-hexane. Then, 100 mL of distilled water containing 0.5% (w/v) sodium chloride was added to the mixture. After continuous manual shaking, the phase separation was achieved, so the upper layer was removed and transferred into a 50 mL volumetric flask. The absorption spectrum of the water insoluble compounds was recorded at 272 nm using a photodiode array spectrophotometer (Model 8453, Agilent Technologies Inc., Waldbronn, Germany). A standard curve of astaxanthin was performed according to Tolasa et al. (2005); 0.5, 1.0, 1.5, 2.0, and 3.0 mL of stock solution of astaxanthin (0.2 mg/mL) were diluted to 10 mL with *n*-hexane and the

Table 1
Average content of moisture (g/100 g), NaCl (g/100 g, dry basis), astaxanthin (μg/g, dry basis), fat (g/100 g, dry basis), cholesterol (mg/g lipids), single and total COPs (μg/g lipids) in raw and boiled shrimp.

	Raw	Boiled
Moisture	78.0 ± 0.4[a]	67.5 ± 0.9[b]
NaCl content	1.1 ± 0.1[a]	4.0 ± 0.4[b]
Astaxanthin content	120.2 ± 3.5[a]	102.9 ± 0.9[b]
Fat content	10.4 ± 1.9[a]	10.7 ± 1.0[a]
Cholesterol	62.9 ± 1.0[a]	62.7 ± 4.2[a]
7α-HC	5.8 ± 2.6[a]	7.8 ± 3.5[a]
7β-HC	4.7 ± 3.1[a]	5.8 ± 4.6[a]
β-CE	4.7 ± 2.1[a]	4.4 ± 2.7[a]
α-CE	12.9 ± 7.4[a]	14.0 ± 6.9[a]
25 HC	3.3 ± 2.0[a]	4.9 ± 2.9[a]
7-KC	7.5 ± 4.3[a]	6.8 ± 5.4[a]
Total COPs	38.9 ± 21.5[a]	43.6 ± 26.0[a]

Each entry represents the means ± standard deviation of three replicates. Means in the same row followed by different lower-case letters are significantly different ($p < 0.05$).

absorption spectrum of each solution was recorded at 272 nm. The standard curve was obtained by plotting the concentration against absorbance. An elevated correlation coefficient was obtained and the content of astaxanthin was calculated from the regression equation of the standard curve ($y = 0.2066x - 0.0271$; $R^2 = 0.9994$).

2.6.10. Kinetic analysis of astaxanthin degradation

Degradation of astaxanthin has been found to follow a first order kinetic reaction (Niamnuy, Devahastin, Soponronnarit, & Raghavan, 2008a). The first-order kinetic model is represented as:

$$ln\left(\frac{C}{C_0}\right) = -kt \quad (3)$$

where C_0 is the initial concentration of astaxathin (mg/100 g shrimp D. B.); C is the concentration of astaxanthin (mg/100 g shrimp d.b.) at any time t; k is the rate constant (h^{-1} or day^{-1}) and t is time (h or day). A plot of $ln\,(C/C_0)$ vs. t was constructed to estimate the value of k by linear regression.

2.7. Statistical analysis

The data are reported as mean values of three replicates for each analytical determination. Means and standard deviations are reported in Tables 1–4. One-way ANOVA and Tukey's honest significant multiple comparison test were used to determine statistical differences between samples ($p < 0.05$). Statistical analysis of the data was performed using the STATISTICA v. 5.0 software (Statsoft, Tulsa, OK, USA).

3. Results and discussion

3.1. Boiling in brine

Boiling of shrimp in brine is an important step, because it reduces the microbial load to acceptable levels and improves the sensory properties of products (Niamnuy et al., 2007). In this study, a marked decrease in the moisture content was observed after cooking (Table 1). A similar observation was made by Niamnuy, Devahastin, and Soponronnarit (2008b)and Unlusayin, Erdial, Gumus and Gulyavuz (2010)after boiling shrimps in brine. During cooking, heat induces protein denaturation and aggregation, causing actin, myosin and collagen shrinkage, which leads to water expulsion from muscle cells and the consequent water loss. During boiling, protein content also decreased. Water plus protein losses are called cooking loss (Niamnuy et al., 2008b). In this study, cooking loss observed was 32%, which is higher than losses reported by Niamnuy et al. (2007)for boiled shrimp in salt solution (4%, w/v) for 7 min. The greater cooking loss we found can be attributed to the longer boiling time (15 min vs. 7 min). On the other hand, the NaCl content in shrimp showed a significant increase after boiling in brine (Table 1); this is consistent with data from other studies (Niamnuy et al. 2008b; Unlusayin, Erdial, Gumus, & Gulyavuz, 2010).

After boiling, the content of astaxanthinin cooked shrimp was significantly lower than in raw shrimp (Table 1). This decrease could be related to denaturation and partial solubilization of the carotenoprotein complex. In this regard, Niamnuy et al. (2008b) found a significant decrease in proteins content (myofibrillar, sarcoplasmic and stroma) in boiled shrimp, suggesting that part of these proteins could have been dissolved in the salt solution.

No significant change was observed in fat and cholesterol content in shrimp during boiling (Table 1). A similar behaviour had previously been reported when shrimps were boiled in brine (Unlusayin et al., 2010). No significant changes were observed on the single fatty acids by boiling in brine. PUFA are the most abundant fatty acid class in shrimp, followed by SFA and MUFA (Table 2). The fatty acid profile found in this work agrees with that reported elsewhere (Sriket et al., 2007).

Several COPs commonly present in foods were found in raw shrimp samples (Table 1). The total content of COPs was 38.9 μg/g lipid, which is similar to that found (33 μg/g lipid) in fresh shrimp (*Penaeus vannamei*) marketed in Spain (Echarte, Conchillo,

Table 2
Effect of boiling, solar drying and storage time on the main fatty acids in dried salted shrimp. Fatty acids are expressed as % of total fatty acids.

	Fatty acid		SFA	MUFA	PUFA
	20:5 *n*−3 (EPA)	22:6 *n*−3 (DHA)			
Raw	9.2 ± 0.9[ab]	6.1 ± 0.5[ab]	33.3 ± 3.1[cde]	29.5 ± 0.0[a]	37.2 ± 3.0[abcd]
Boiled	9.6 ± 0.1[a]	7.0 ± 0.3[a]	30.9 ± 0.4[e]	27.1 ± 0.8[a]	42.0 ± 0.7[a]
Solar drying time (h)					
8	9.1 ± 1.2[abc]	5.6 ± 0.7[bcd]	33.5 ± 2.3[bcde]	27.5 ± 1.4[a]	39.0 ± 2.4[abc]
16	8.9 ± 0.3[abc]	5.9 ± 0.1[abc]	32.8 ± 1.7[de]	28.5 ± 1.2[a]	38.7 ± 2.1[abcd]
24	8.2 ± 0.3[abc]	5.4 ± 0.1[bcd]	34.5 ± 1.4[abcde]	28.8 ± 1.7[a]	36.7 ± 1.0[abcd]
32	8.8 ± 0.4[abc]	5.4 ± 0.4[bcd]	34.5 ± 0.6[abcde]	25.9 ± 2.1[a]	39.6 ± 2.5[ab]
Storage time (days)					
15	8.0 ± 0.6[abc]	4.5 ± 0.4[d]	36.0 ± 2.6[abcd]	27.2 ± 4.6[a]	36.8 ± 4.2[abcd]
30	7.8 ± 0.4[bc]	5.0 ± 0.3[bcd]	37.7 ± 0.4[abc]	29.5 ± 0.8[a]	32.8 ± 0.6[de]
45	7.5 ± 0.1[bc]	4.8 ± 0.2[cd]	38.2 ± 0.5[a]	28.3 ± 1.3[a]	33.5 ± 0.9[cde]
60	7.6 ± 0.7[bc]	4.7 ± 0.3 d	38.0 ± 0.3[ab]	28.0 ± 0.3[a]	34.0 ± 0.3[bcde]
75	8.2 ± 0.6[abc]	5.3 ± 0.4[bcd]	37.7 ± 1.4[abc]	27.2 ± 2.5[a]	35.1 ± 1.3[bcde]
90	7.3 ± 0.6[c]	4.5 ± 0.3[d]	38.5 ± 0.0[a]	31.2 ± 0.8[a]	30.3 ± 0.7[e]

Each entry represents the means ± standard deviation of three replicates. Mean in the same column followed by different letter are significantly different ($p < 0.05$).

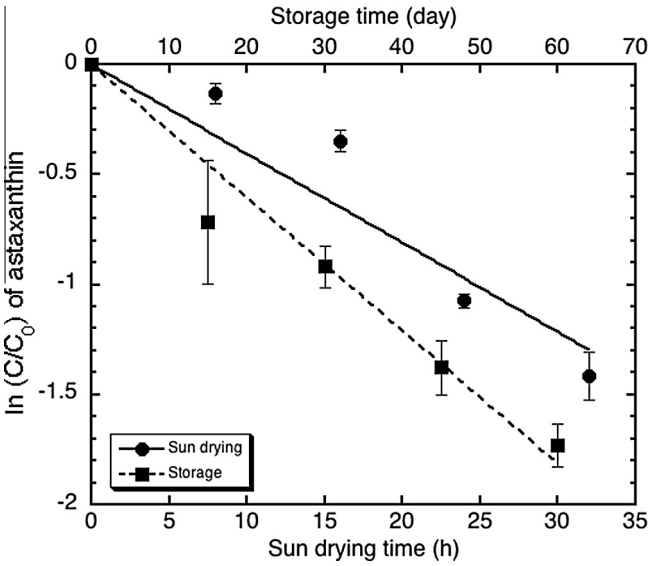

Fig. 1. First order kinetic plot of astaxanthin degradation of boiled shrimp during sun drying and storage. Abbreviations: ●, sun drying (mean values ± standard deviation of three replicates); —, sun drying best fit; ■ storage (mean values ± standard deviation of three replicates); - - -, storage best fit.

Ansorena & Astiasaran, 2005). Main COPs found in raw shrimp were 7α-HC, 7β-HC, α-CE, β-CE, 7-KC and 25-HC. This profile is consistent with reports by Echarte, Conchillo, Ansorena, and Astiasarán (2005) with the exception of CT, which was not detected in the present study. The occurrence of COPs in raw shrimp could be attributed to the oxidation of cholesterol by autoxidation and enzymatic mechanisms. Considering the above, the temperature at which the shrimp are handled and the time elapsed from capture to marketing is very important. Echarte et al. (2005) indicated that freezing shrimp after capture is a way to minimize COPs formation in the product during marketing.

No significant changes were observed in the content of the different COPs in raw and boiled shrimps (Table 1). The temperature reached during sample boiling was 100 °C. Several researchers have established that cholesterol is stable at 100 °C, but some COPs begin to form when stored with polyunsaturated lipids for long periods of time (Ohshima, 2002). The lipid fraction of shrimp had a high content of PUFA (Table 2), which could have favoured cholesterol oxidation during boiling. However, apparently the boiling conditions (100 °C for 15 min) were not enough to generate a substantial amount of COPs in cooked shrimp. It might be also possible that, during boiling, part of COPs also decomposed and/or reacted with other molecules (such as proteins), generating compounds that could not be detected under the analytical conditions used (Olkkonen & Hynynen, 2009).

3.2. Sun drying of boiled shrimp

Direct sun drying is an inexpensive method of food preservation that is still used in different regions. In this study, moisture content in cooked shrimp decreased during sun drying, reaching 13.9% after 4 days. Temperature ranged from 28.9 to 35.7 °C during sun drying and the relative humidity varied from 70.7% to 96.4%.

Astaxanthin content in cooked shrimp decreased by 75% after four days of direct sun drying, reaching 25.0 ± 2.7 μg/g. This astaxanthin content is much lower than that obtained in a jet spouted bed drier at 80, 100 and 120 °C (Niamnuy et al., 2008a). These latter authors noted that if the drying temperature decreased, the retained astaxanthin content was smaller, indicating greater degradation of the carotenoid. It was concluded that lower temperatures require longer drying times, which promote the hydrolysis of esterified astaxanthin, yielding free astaxanthin that is more susceptible to oxidation. Sun drying of cooked shrimp requires long periods of time, which could lead to greater release of astaxanthin from its esterified form. Additionally, the susceptibility of free astaxanthin to photobleaching has been documented (Christophersen, Jun, Jorgensen, & Skibsted, 1991). This would explain the rather high degradation rate of this carotenoid observed during sun drying of shrimp.

Astaxanthin degradation followed first order kinetics during sun drying of shrimp (Fig. 1), and the rate constant (k) obtained was $4.05 \pm 0.24 \times 10^{-2}$ (h^{-1}) with an R^2 of 0.95. Thermal degradation of astaxanthin has been studied in several model systems (Niamnuy et al., 2008a). However, photochemical reactions, such as photolysis and photosensitized oxidation, are also responsible for the loss of astaxanthin during sun drying of shrimp.

The decline in astaxanthin content in shrimp during sun drying mainly affects the product colour. However, considering the high antioxidant activity of astaxanthin (Naguib, 2000), the degradation of this carotenoid could also have an affect on the oxidative stability of fatty acids and cholesterol.

Significant decreases in the PUFA content have been reported during sun drying of some fish species (Salah, Nawzet, Mourad, Mohsen, & Monia, 2010). However, our data show only a slight declining trend in EPA, DHA and total PUFA (Table 2).The lipid fraction of shrimp is mainly formed by phospholipids (72–74%), and triacylglycerols (16%) (Sriket et al., 2007), and most PUFA are esterified to phospholipids rather than to triacylglycerols. It has been established that marine phospholipids are more resistant towards oxidation than bulk fish oil (mostly triacylglycerols) from the same source (Henna-Lu, Nielsen, Timm-Heinrich, & Jacobsen, 2011). This could explain the insignificant changes observed in the content of EPA and DHA during sun drying.

Cholesterol is susceptible to oxidation during food processing and produces different COPs (Otaegui-Arrazola et al., 2010). In the present survey, a dramatic increase in the total content of COPs in shrimp was observed during sun drying, reaching a total content of 372.9 ± 16.3 μg/g of lipids after 32 h of direct sunlight. Direct exposure to UV and VIS light during sun drying of shrimp could be one of the main factors that caused oxidation of cholesterol in the samples (photo-oxidation). Several research findings in foods and model systems have shown that photo-oxidation of cholesterol is an important mechanism to consider, especially when this sterol is found together with PUFA and photosensitizers (Chien, Lu, Hu, & Chen, 2003; Lercker & Rodriguez-Estrada, 2002). Other factors, such as temperature reached during drying, the presence of oxygen and pro-oxidant compounds, could also contribute to cholesterol oxidation in shrimp.

Fig. 2 shows the kinetics of COPs identified during sun drying of shrimp. The COPs formed in greater amounts were 7α-HC and 7β-HC, followed by α-CE, β-CE and 7-KC. The mechanisms of cholesterol oxidation have been addressed by several researchers under different conditions (Chien et al., 2003; Lercker, 2002). Cholesterol oxidation can be initiated by hydrogen abstraction at C-7, followed by addition of an oxygen molecule, which leads to formation of 7α-OOH and 7β-OOH. These hydroperoxides can be formed by high temperatures, direct exposure to UV light or by photo-oxidation through a type I reaction (Chien et al., 2003). Moreover, singlet oxygen formed by triplet sensitizer-ground state oxygen interaction (type II reaction) can react with cholesterol via a non-radical mechanism, producing mainly 5α-OOH. The 5α-OOH tends to convert rapidly into 7α-OOH, which later isomerizes to 7β-OOH (Chien et al., 2003; Lercker, 2002). Both 7α-OOH and 7β-OOH could produce 7α-OH and 7β-OH by reduction or 7-KC by dehydration. Later, 7α-HC and 7β-HC could produce 7-KC by dehydrogenation (Chien, Hsu, & Chen, 2006). During sun drying of shrimp, a greater formation of 7-HCs (α and β) was observed as compared to 7-KC (Fig. 2),

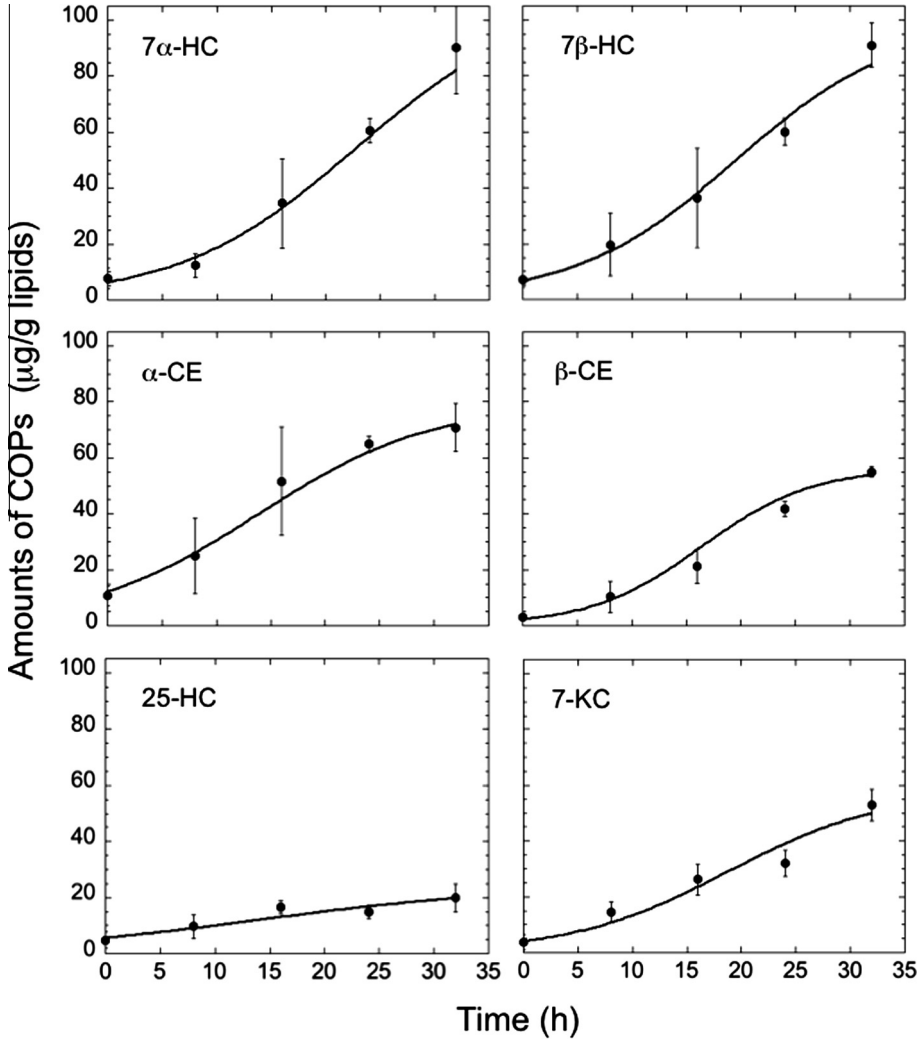

Fig. 2. Formation of major COPs in salted dried shrimp during sun drying. Abbreviations: ●, mean values ± standard deviation of three replicates; —, best fit.

which indicates the equilibrium of the dysmutation reaction of hydroperoxides favour their conversion into the hydroxyl derivatives. As observed for hydroperoxides, 7α-HC is commonly found at lower concentrations levels than its corresponding epimer, as the α is less favoured from a thermodynamic standpoint (Lercker, 2002). However the presence of photosensitizers may provide an environment for promoting photo-oxidation, and thus the formation of 7α-HC is favored (Chien et al., 2003). In our study, 7α-HC production was similar to 7β-HC possibly due to an increase in the formation of 7α-OOH through a photo-oxidative pathway.

Another pathway of cholesterol oxidation is carried out when a hydroperoxyl radical and cholesterol interact to produce α-CE y β-CE through a mechanism similar to that observed in the oxidation of MUFA (Lercker, 2002). Conversely, carbons 20 and 25 of the side-chain of cholesterol are also susceptible to oxidation. Initially hydroperoxides are formed, which produce the corresponding hydroxides by reduction. 25-HC is the most common side-chain COPs found in foods; however, its presence is always less than 7-HC (α and β), 7-KC and CE (α and β) (Ohshima, 2002). Fig. 2 shows a smaller formation of 25-HC than the rest of COPs during sun drying of shrimp.

Table 3 shows the kinetic parameters for the formation of different COPs generated during sun drying of cooked shrimp. Correlation coefficients obtained for the best fitting curves (except for 25-HC) ranged from 0.80 to 0.92, indicating an overall good agreement with the experimental data. The kinetic curves for 7α-HC, 7β-HC, α-CE and β-CE formation showed an induction period of ca. 8 h, where the formation rate was small, followed by a period where it markedly increased (Fig. 2). Other researchers report similar trends, and found different induction times in different model systems (Chien et al., 2006; Ohshima, Li, & Koizumi, 1993). Ohshima et al. (1993) observed a drastic increase in the formation of COPs when more than 50% of EPA and DHA were degraded within the model system. Moreover, Chien et al. (2006) observed an increase in the induction period during COPs formation, due to the presence of quercetin, a well-known phenolic antioxidant.

Table 3
Modelled kinetic parameters for formation of 7α-HC, 7β-HC, β-CE, α-CE, 25-HC, 7-KC and total COPs in boiled shrimp during sun drying.

COPs	C_0 (mg/g lipids)	$k \times 10^{-2}$ (h^{-1})	R^2
7α-HC	6.2	12.3 ± 1.1	0.92
7β-HC	6.6	13.6 ± 1.2	0.91
β-CE	2.2	19.7 ± 1.9	0.90
α-CE	12.0	12.4 ± 1.8	0.80
25-HC	5.6	8.4 ± 1.9	0.61
7-KC	4.0	13.9 ± 1.6	0.87
Total COPs	36.5	12.6 ± 1.0	0.93

Table 4
Average content of moisture (g/100 g), astaxanthin (μg/g, dry basis), fat (g/100 g, dry basis), single and total COPs (μg/g lipids) in dried shrimp during storage.

	Storage time (days)						
	0	15	30	45	60	75	90
Moisture	13.9 ± 0.6^{b}	13.8 ± 0.5^{b}	14.9 ± 0.2^{a}	13.3 ± 0.1^{b}	15.1 ± 0.1^{a}	14.9 ± 0.1^{a}	14.9 ± 0.2^{a}
Astaxanthin content	25.0 ± 2.7^{a}	12.5 ± 3.3^{b}	10.0 ± 1.0^{bc}	6.4 ± 0.8^{cd}	4.5 ± 0.4^{d}	4.8 ± 0.4^{d}	4.3 ± 0.6^{d}
Fat content	10.6 ± 0.7^{c}	12.1 ± 1.5^{bc}	13.3 ± 0.2^{bc}	14.5 ± 1.2^{ab}	12.8 ± 1.9^{bc}	17.1 ± 0.8^{a}	11.6 ± 0.0^{bc}
7α-HC	90.3 ± 16.5^{abA}	175.0 ± 32.3^{aA}	79.5 ± 32.3^{bAB}	79.5 ± 31.8^{bA}	160.6 ± 10.0^{abAB}	163.8 ± 50.9^{abA}	135.6 ± 21.1^{abB}
7β-HC	91.0 ± 7.8^{cA}	174.2 ± 23.7^{abcA}	117.0 ± 11.6^{bcA}	115.9 ± 14.8^{bcA}	222.0 ± 29.1^{aA}	191.7 ± 67.4^{abA}	221.9 ± 15.9^{aA}
β-CE	55.0 ± 1.8^{bB}	102.7 ± 15.2^{abB}	55.7 ± 35.0^{bBC}	90.0 ± 4.9^{abA}	115.4 ± 32.8^{abB}	138.0 ± 45.3^{aABC}	145.3 ± 18.9^{aB}
α-CE	65.1 ± 4.9^{cB}	108.8 ± 4.4^{abB}	74.8 ± 14.6^{bcAB}	90.4 ± 6.7^{abcA}	117.5 ± 25.4^{abB}	97.8 ± 18.0^{abcABC}	118.5 ± 20.4^{aB}
CT	2.0 ± 3.5^{cC}	10.5 ± 1.7^{bC}	10.0 ± 2.7^{bC}	10.2 ± 3.5^{bB}	15.4 ± 2.6^{abC}	16.5 ± 4.3^{abC}	21.6 ± 0.4^{aC}
25 HC	16.6 ± 6.4^{bC}	22.5 ± 2.5^{abC}	28.9 ± 7.2^{abBC}	23.2 ± 4.3^{abB}	31.8 ± 6.8^{aC}	30.6 ± 2.5^{abBC}	30.1 ± 4.2^{abC}
7-KC	52.9 ± 5.8^{dB}	112.3 ± 3.0^{bcdB}	74.3 ± 14.5^{cdAB}	87.0 ± 8.2 ^{cdA}	187.6 ± 37.2^{abA}	149.8 ± 62.9^{abAB}	213.6 ± 16.9^{aA}
Total COPs	372.9 ± 16.3^{d}	705.9 ± 72.5^{abc}	440.3 ± 81.2^{cd}	496.1 ± 63.8^{bcd}	850.2 ± 57.5^{a}	788.0 ± 246.1^{ab}	886.6 ± 97.9^{a}

Each entry represents the means ± standard deviation of three replicates. Entries bearing different lower-case letters (a–d) in the same row are significantly different ($p < 0.05$). Entries bearing different capital letters (A–C) in the same column are significantly different ($p < 0.05$).

3.3. Storage

Astaxanthin content continued to decline during storage of salted dried shrimp. After 60 days, 74% of the astaxanthin level measured at the beginning of storage had already degraded, resulting in an astaxanthin content equal to 4.5 ± 0.4 (μg/g); no significant further changes were found until the end of storage (Table 4).

Astaxanthin degradation during storage followed first order kinetics (Fig. 1) and the rate constant (k) obtained was $3.02 \pm 0.13 \times 10^{2}$ h^{-1} (21.14×10^{2} week^{-1}) with a R^2 of 0.98. Niamnuy et al. (2008a) reported a k value of 4.99×10^{2} (week^{-1}) during storage of samples that had been dried at 80 °C and kept at 25 °C for 6 weeks. The high rate of astaxanthin degradation observed during the storage of sundried shrimps can be attributed to an elevated accumulation of free astaxanthin derived from the hydrolysis of their esterified form during sun drying.

Likewise, a marked decline of PUFA was observed during storage (Table 2), especially EPA and DHA. PUFAs are susceptible to oxidation even under mild environmental conditions, generating lipid peroxyl radicals that may react with cholesterol to form cholesterol peroxyl radicals, which are precursors of several COPs (Ohshima, 2002). Through this mechanism, the presence of PUFAs in the lipid fraction of salted dried shrimp and the storage conditions considered in the present study could have contributed to the oxidation of cholesterol during storage. COPs formation in dried salted shrimp continued along the 90-day storage (Table 4). Total content of COPs measured was 886.6 ± 97.9 μg/g of lipids (10.28 ± 1.1 mg/100 g of dried shrimp) after storage. This amount corresponds to more than twice the COPs contained in the samples at the beginning of storage. Soto-Rodríguez et al. (2008) analyzed commercial samples of dried salted shrimp from Mexico and found a total content of COPs that varied from 13.0 to 25.4 mg/100 g of dried shrimp. On the other hand, dried salted shrimp from different seasons and regions in Brazil showed a total content of COPs from 0.674 to 5.487 mg/100 g of dried shrimp (Sampaio et al., 2006). Differences on the total content of COPs from previous reports and the current study could be ascribed to several factors, such as the sample origin (species, capture season), processing, and storage conditions prior to analysis. Storage time is a very important variable in the formation and accumulation of COPs in the dried shrimp, as shown in Table 4. Therefore, a higher content of COPs was observed as storage time increased. Although the toxic effects of COPs have been studied in recent years, it has been established that more research is required to better ascertain the toxicity levels of particular COPs (Otaegui-Arrazola et al., 2010). However, according to the threshold of toxicological concern (TTC) for unclassified compounds (0.15 μg per person per day) (Kroes et al., 2004), the consumption of dried salted shrimp with COPs levels as those found in the present study could be considered as a significant health risk.

All COPs monitored showed a significant increase over the 90-day storage (Table 4). 7β-HC, 7-KC, 7α-HC, β-CE and α-CE were formed in greater quantities during storage, while 25-HC and CT were found only in small amounts. CT was detected only during the storage of dried shrimp. This COP is formed through the hydration of α-CE and β-CE and it is usually found in smaller proportions than other COPs (Lercker, 2002).

In contrast to sun drying, 7-KC was formed in similar amount to 7β-HC and 7α-HC during storage (Table 4). The greater formation of 7-KC during storage could occur, because during this period 7-KC was produced by dehydration of 7α-OOH and 7β-OOH plus the dehydrogenation of 7β-HC and 7α-HC previously accumulated. The COPs profile found in dried salted shrimp at the end of storage was similar to that reported by Soto-Rodríguez et al. (2008). An important factor related to the formation of COPs during storage of foods is the coexistence of triacylglycerols rich in PUFA. Ohshima et al. (1993) noted that cholesterol oxidation took place at 25 °C when fish oil was present in the system, and mainly 7β-HC, 7-KC, β-EC, α-EC and CT were formed.

4. Conclusion

This study indicates an elevated formation of COPs during direct sun drying and further storage of dried salted shrimp. Similarly, a significant loss of astaxanthin was observed mainly during sun drying. Considering the high antioxidant activity of astaxanthin, oxidation of cholesterol in shrimp could be related to the degradation of astaxanthin. The high formation of COPs and astaxanthin degradation observed during traditional processing of dried salted shrimp suggest that both processing and storage conditions should be re-assessed, in order to decrease lipid oxidation and to improve the overall quality of the product.

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