Research Article

Effect of cooking conditions on cholesterol oxidation and astaxanthin in dried salted shrimp

Josafat A. Hernández-Becerra[1, 2], Angélica A. Ochoa-Flores[1, 3], Ida Soto-Rodriguez[4], Maria T. Rodriguez-Estrada[5] and Hugo S. García[1]

[1] UNIDA, Instituto Tecnológico de Veracruz, Veracruz, Veracruz, Mexico
[2] División de Procesos Industriales, Universidad Tecnológica de Tabasco, Villahermosa, Tabasco, Mexico
[3] División Académica de Ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco, Mexico
[4] Facultad de Bioanálisis, Universidad Veracruzana. Carmen Serdán s/n, Veracruz, Veracruz, Mexico
[5] Dipartimento di Scienze e Tecnologie Agro-Alimentari, Alma Mater Studiorum-Università di Bologna, Bologna, Italy

Dried salted shrimp is a product made from raw shrimps, which are usually cooked and dried under direct sunlight. Brine cooking is an important step during the production of dried salted shrimp as it promotes changes that affect the product's end quality. The aim of this study was to evaluate the effect of brine concentration and boiling time on cholesterol oxidation products (COPs) formation and the concomitant changes in astaxanthin content and fatty acid profile in shrimp during cooking, sun drying, and storage. Boiling conditions did not affect COPs formation in shrimp after cooking. However, increased brine concentration and boiling time promoted high astaxanthin retention in cooked shrimp. During the first 24 h of sun drying, COPs formation in dried salted shrimp was influenced by the interaction between brine concentration and boiling time; in fact, the lowest COPs levels were observed in samples boiled at low brine concentration and short cooking times, as well as those boiled in brine with high salt concentration and long cooking times. Most astaxanthin (ca. 78%) present in cooked shrimp was degraded during solar drying. During storage, PUFA decreased, and a concomitant astaxanthin degradation and COPs formation in dried salted shrimp were observed. Neither boiling time nor storage at dark of dried salted shrimp prevented these changes.

Practical applications: The content of oxysterols in cooked dried shrimps can be minimized by optimizing the processing conditions to minimize the oxidation. This is useful for processors and consumers to reduce the intake of these toxic forms of cholesterol.

Keywords: Astaxanthin / Cholesterol oxidation / Cooking conditions / Shrimp / Sun drying

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1 Introduction

Shrimp trade is one of the world's most important economic activities of the fishery industry. A product from fresh shrimp processing with documented uses in the traditional cuisine of various countries is dried salted shrimp. This product is prepared by boiling raw shrimp in brine, dried under direct sunlight for 3–5 days, and packaged in plastic bags for bulk storage [1]. Brine cooking is a key step, because it reduces the microbial load in shrimp down to acceptable levels and improves the sensory properties of the product [2]. During boiling, heat induces protein denaturation and aggregation, which in turn causes changes in the microstructure of shrimp muscle and decreases both moisture and protein contents, with a concomitant increase in salt content. Changes observed were dependent on the boiling conditions, particularly brine concentration and boiling time [2–4]. No significant changes have been noted in the lipid content as a result of boiling in brine [4]. However, the observed alterations in the structural and functional proteins of shrimp and the salt incorporated into the product could cause alterations in cell

Correspondence: Prof. Hugo S. García, UNIDA, Instituto Tecnológico de Veracruz, Av. Miguel Ángel de Quevedo # 2779. Col. Formando Hogar, Veracruz, Veracruz 91897, Mexico
E-mail: hsgarcia@itver.edu.mx
Fax: +52 229 9345701x201

Abbreviations: COPs, cholesterol oxidation products; **d.b.,** dry basis; **IS,** internal standard; **α-CE,** 5,6α-epoxycholesterol; **β-CE,** 5,6β-epoxycholesterol; **7α-HC,** 7α-hydroxycholesterol; **7β-HC,** 7β-hydroxycholesterol; **25-HC,** 25-hydroxycholesterol; **7α-OOH,** 7α-hydroperoxycholesterol; **7β-OOH,** 7β-hydroperoxycholesterol; **7-KC,** 7-ketocholesterol

membranes, thus promoting oxidative processes in the lipid fractions [5].

Cholesterol is an important component of shrimp's lipid fraction. It is highly susceptible to oxidation, leading to the formation of cholesterol oxidation products (COPs). COPs have proven adverse effects on human health, such as cytotoxicity, mutagenicity, and carcinogenicity [6]. Moreover, COPs are involved in the initiation and progression of several chronic diseases, such as atherosclerosis, neurodegenerative disorders, diabetes, and kidney failure [6]. 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, time, and exposure to UV/VIS light) [7]. 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 [8, 9]. However, information about the formation of COPs in dried salted shrimp and the effect of boiling conditions on the COPs content of shrimp during processing and storage, is limited. The aim of this study was to evaluate the effect of brine concentration and boiling time on COPs formation and the concomitant changes in astaxanthin content and fatty acid profile in shrimp during cooking, sun drying and storage.

2 Materials and methods

2.1 Materials

Fresh white shrimps (*Litopenaeus vannamei*) were obtained from a local seafood market in Veracruz (Mexico). The average weight of individual shrimps was 19.4 ± 2.8 g (45–60 shrimps/kg); 4.5 kg of raw shrimps were used in the study. After grading and washing with tap water, raw shrimps were divided into five groups and subjected to different cooking treatments in triplicate. Prior to their preparation, shrimps were maintained at 2°C in an ice chest filled with crushed ice (about 3 h).

2.2 Boiling in brine

Raw whole shrimps were kept at room temperature for 10 min before cooking in brine. Cooking was performed in a 25 cm diameter stainless steel pot. The boiling conditions were as follows: concentration of salt in brine of 5 or 20% w/w, boiling times of 5 or 25 min. An additional treatment of 12.5% w/w of brine concentration and 15 min of boiling time was included. In all treatments, the shrimp to brine ratio was 1:4 on weight basis. After boiling, shrimps were separated from the brine and allowed to reach room temperature. All boiling experiments were performed in triplicate.

2.3 Sun drying

Cooked shrimps were spread on a plastic mesh and exposed to direct sunlight for periods of 8 h every day for 5 consecutive days. During sun drying, each shrimp was flipped every 2 h, to ensure similar exposure to sunlight on both sides.

2.4 Storage

Sun-dried salted shrimp prepared with 5% brine for 5 and 25 min were split into two groups. The first group was packed in transparent polypropylene boxes (nine shrimps/box), under normal atmosphere and wrapped with aluminum foil to protect the shrimp from light during storage. The second group was packed in the same way but without aluminum foil. All boxes containing shrimp were kept for 150 days at room temperature (18–28°C).

2.5 Sampling

Samples of each treatment were taken during processing and storage as follows: (1) before boiling in brine (whole raw shrimp), (2) after boiling in brine (boiled shrimp), (3) after 24 and 40 h of sun drying, and (4) after 75 and 150 days of storage.

2.6 Reagents and Chemicals

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

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 by volume.

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

2.7 Methods

2.7.1 Moisture analysis

Moisture content was determined by the AOAC method 950.46 [10].

2.7.2 Sodium chloride analysis

Sodium chloride concentration was determined by the AOAC method 935.47 [11].

2.7.3 Lipid extraction

Lipid extraction was performed according to Boselli et al. [12]. About 16 g dry basis (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 produce phase separation. The lower phase was collected and dried in a vacuum evaporator. The fat content was determined gravimetrically. The lipid extract was stored at −20°C in *n*-hexane:isopropanol (3:2 v/v) until analyzed.

2.7.4 Fatty acids analysis by gas chromatography

FAME were prepared according to Soto-Rodríguez et al. [9]. 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 microliter of the methylated preparation was injected into a HP-6890 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 are expressed as area percentage with respect to total FAME area.

2.7.5 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 ISs for the quantification of COPs and cholesterol, respectively. The sample was then dried under nitrogen flow and added with 10 mL of 1 N KOH solution in methanol, wrapped with aluminum foil and shaken for 18 h, in order to produce saponification [9]. For 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 N KOH and 5 mL of saturated NaCl. The extracts were dried over anhydrous sodium sulfate. 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.

2.7.6 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 by volume) at 40°C for 15 min; subsequently, it was dried under nitrogen flow and dissolved in 100 μL of *n*-hexane [13]. One microliter of the silylated preparation was injected into an Agilent 7820 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA), equipped with a split–splitless injector and 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. The injector and FID temperatures were both set at 325°C.

Total cholesterol was quantified by the IS method, using 5α-cholestane. Peak identification of cholesterol was carried out by comparing the peak retention time with that of cholesterol standard and by spiking the samples with a small amount of cholesterol standard. Quantification of cholesterol was performed by using relative response factors [14] which were calculated using standard solutions with known concentrations of cholesterol and 5α-cholestane.

2.7.7 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 [15]. 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 [13], 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 IS method, using betulin as IS. Peak identification of COPs was performed by comparing the peak retention times with those of the corresponding 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 [14].

2.7.8 Astaxanthin analysis

Astaxanthin content was determined according to the method of Tolasa et al. [16]. About 10 g of sample were extracted three times with 40 mL of 0.05% BHT solution (in acetone) with an Ultra-Turrax IKA® T25 Digital homogenizer (Ika-Werke, Staufen, Germany) for 2 min. Samples were kept cool by immersing in crushed ice during homogenization to avoid over-heating. After extraction, samples were centrifuged at 4000*g* and 4°C for 5 min. To separate the water insoluble compounds, the acetone extracts from 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 were 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 472 nm using a photodiode array spectrophotometer (Model 8453, Agilent Technologies, Inc., Waldbronn, Germany). A standard curve of astaxanthin was prepared according to Tolasa et al. [16]; 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 absorption spectrum of each solution was recorded at 472 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 ($y = 0.2066x - 0.0271$; $R^2 = 0.9994$).

2.7.9 Experimental design and data analysis

A factorial experimental design at two levels and one central point was planned and conducted in triplicate to study the influence of concentration of salt in brine and boiling time on moisture, sodium chloride, astaxanthin, COPs and fatty acids content during process and storage of dried salted shrimp. Data were analyzed using the STATISTICA v. 6.0 software (Statsoft, Tulsa, OK). First-order coefficients were generated by regression and the data were fitted by multiple regression. The fit of the model was evaluated by coefficients of determination (R^2) and ANOVA. The linear response surface model was fitted to the following equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 \quad (1)$$

where Y is the response variables, X_1 and X_2 are the independent variables (concentration of salt in brine and boiling time), β_0 the intercept, β_1 and β_2 are the coefficients of the first-order model and β_{12} is the coefficient of the linear model for the interaction between factors. Means and SDs are reported in Tables 1, 4, 5, and 6. Differences between mean values were established using Tukey's honest significance multiple comparison test ($p < 0.05$).

3 Results and discussion

3.1 Boiling in brine

Brine cooking is a step where important modifications occur in shrimp [2]. In the present study, significant changes in moisture, NaCl, and astaxanthin content were observed in boiled shrimp after cooking (Table 1). Such changes were significantly dependent on the boiling conditions used

Table 1. Effect of different boiling conditions on moisture content, astaxanthin and NaCl in shrimp after cooking and sun drying

Concentration of NaCl in brine (% w/w)	Boiling time (min)	Moisture (g/100 g)	Astaxanthin (μg/g d.b.)	NaCl (g/100 g d.b.)
Raw shrimp				
0	0	75.3 ± 0.1 a	248.2 ± 11.2 a	1.4 ± 0.5 f
Boiled shrimp				
5	5	69.7 ± 0.5 b	103.3 ± 2.1 d	3.0 ± 0.1 e
5	25	69.0 ± 0.2 b	120.7 ± 1.2 cd	6.9 ± 0.3 d
20	5	64.3 ± 0.1 d	137.4 ± 1.5 bc	10.1 ± 0.4 c
20	25	60.5 ± 0.2 e	156.2 ± 3.3 b	20.6 ± 0.2 a
12.5	15	66.2 ± 0.0 c	127.8 ± 7.2 cd	14.1 ± 0.4 b
Boiled shrimp after 24 h of sun drying				
5	5	15.7 ± 0.2 gh	43.4 ± 3.5 ef	
5	25	16.1 ± 0.1 fg	42.9 ± 0.3 ef	
20	5	15.2 ± 0.7 h	29.4 ± 4.1 f	
20	25	16.7 ± 0.7 f	38.1 ± 1.3 ef	
12.5	15	16.7 ± 0.4 f	57.4 ± 12.4 e	
Boiled shrimp after 40 h of sun drying				
5	5	12.1 ± 0.5 j	24.1 ± 1.1 f	
5	25	13.0 ± 0.6 i	31.2 ± 7.8 f	
20	5	12.1 ± 0.1 j	31.2 ± 0.7 f	
20	25	12.6 ± 0.2 ij	33.2 ± 10.4 ef	
12.5	15	12.0 ± 0.4 j	23.9 ± 7.5 f	

Each entry represents the means ± SD of three replicates. Different superscripts in each column indicate statistical difference ($p < 0.05$).

(Table 2). Table 3 shows the coefficients of the first-order models calculated to estimate the moisture, NaCl and astaxanthin content in cooked shrimp after boiling. Correlation coefficients (R^2) obtained for the best fitting indicate an overall good agreement with the experimental data.

The moisture in boiled shrimp after cooking was affected by the brine concentration, boiling time, and the interaction between both factors (Table 2). Increased brine concentration and boiling time promoted water loss during cooking and thus the moisture content in cooked shrimp was lower

Table 2. *p* Values from the statistical analysis of the effects of brine concentration and boiling time on the different response variables in dried salted shrimp after cooking and sun drying

	P values*								
	After cooking			After 24 h of sun drying			After 40 h of sun drying		
	S	*T*	*S* × *T*	*S*	*T*	*S* × *T*	*S*	*T*	*S* × *T*
Moisture	**0.00**	**0.00**	**0.00**	0.84	**0.03**	0.17	0.59	0.07	0.52
NaCl	**0.00**	**0.00**	**0.00**						
Astaxanthin	**0.00**	**0.01**	0.87	0.08	0.38	0.33	0.38	0.38	0.61
COPs									
7α-HC	0.12	0.57	0.59	0.23	0.79	0.10	0.42	0.47	0.49
7β-HC	0.35	0.59	0.82	0.97	0.79	**0.00**	0.56	0.40	0.34
β-CE	0.25	0.42	0.57	0.29	0.53	**0.01**	0.62	0.72	0.22
α-CE	0.13	0.47	0.39	0.50	0.43	0.13	0.79	0.69	0.15
CT				0.16	0.59	0.91	0.46	0.37	0.30
25 HC	0.85	0.91	0.48	0.47	0.38	0.35	0.25	0.93	0.93
7-KC	0.51	0.38	0.34	0.37	0.99	**0.00**	0.37	0.84	0.24
Total COPs	0.20	0.52	0.54	0.71	0.70	**0.00**	0.53	0.68	0.23
SFA	0.13	0.53	0.93	**0.03**	0.15	**0.02**	0.77	0.11	0.72
MUFA	**0.02**	0.41	0.71	0.24	**0.03**	0.59	0.69	0.77	0.42
PUFA	0.40	0.95	0.05	0.24	0.20	0.49	0.76	0.88	0.38
20:5 n3 (EPA)	**0.02**	0.16	**0.01**	0.14	0.43	0.51	0.98	0.88	0.56
22:6 n3 (DHA)	0.14	0.42	**0.00**	0.29	0.32	0.45	0.86	0.84	0.38

S, concentration of salt in brine; *T*, boiling time; *S* × *T*, interaction; COPs, cholesterol oxidation products; 7α-HC, 7α-hydroxycholesterol; 7β-HC, 7β-hydroxycholesterol; α-CE, 5,6α-epoxycholesterol; β-CE, 5,6β-epoxycholesterol; 7-KC, 7-ketocholesterol; 20-HC, 20-hydroxycholesterol; 25-HC, 25-hydroxycholesterol; CT, cholestanetriol; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
*The concentration of salt and boiling time affect significantly the response variables when $p < 0.05$.
Numbers in bold indicate *p* values less than 0.05.

Table 3. Regression coefficients describing the influence of brine concentration and boiling time on different respond variables in boiled shrimp after cooking and dried salted shrimp after 24 h of sun drying

	Coefficients of lineal model				
	C	*S*	*T*	*S* × *T*	R^2
After cooking					
Moisture	71.4134	−0.3028	0.0174	−0.0104	0.9950
NaCl	1.0014	0.3610	0.0867	0.0219	0.9295
Astaxanthin	87.4229	2.2445	0.8423	0.0051	0.9370
After 24 h of sun drying					
Total COPs	0.4319	0.0412	0.0318	−0.0026	0.8888
7β-HC	0.0656	0.0101	0.0081	−0.0007	0.8160
β-CE	0.0707	0.0089	0.0057	−0.0005	0.7572
7-KC	0.0374	0.0138	0.0102	−0.0008	0.7774

C, constant: *S*, concentration of salt in brine; *T*, boiling time; R^2, coefficient of determination of the models; COPs, cholesterol oxidation products; 7β-HC, 7β-hydroxycholesterol; β-CE, 5,6β-epoxycholesterol; 7-KC, 7-ketocholesterol.

(Fig. 1a). A similar remark was made by Niamnuy et al. [17] and Unlusayin et al. [4] after cooking shrimps in salt solution. Incorporation of NaCl to the shrimp during boiling is also influenced by the cooking conditions. Figure 1b shows that boiling conditions at a high brine concentration and longer cooking times, promotes greater incorporation of NaCl to shrimp. This is consistent with other studies [4, 17].

One of the most noticeable changes in shrimp during cooking is color, which turns to red upon heating. Astaxanthin is the main pigment of crustaceans; it is located mainly in the carapace and head; usually is present in either the free form, esterified or bound to macromolecules, such as proteins (carotene-proteins) [18]. Cleavage of this complex results in a marked color change, due to the release of the carotenoid [19]. Table 1 shows the astaxanthin content in raw and cooked shrimp under our experimental conditions. Boiled shrimp had lower astaxanthin content than the raw shrimp, which indicated a significant loss of this carotenoid as a result of cooking. Aggregates containing astaxanthin probably coming from the integument (carapace and epidermis) of shrimp were most likely released into the cooking medium during boiling. In addition to the above, the possible thermal degradation of astaxanthin during cooking might explain the loss of this carotenoid. The amount of astaxanthin lost in boiled shrimp was affected by the brine concentration and the time boiling (Table 2). A high brine concentration and prolonged cooking time favored greater retention of astaxanthin in cooked shrimp (Fig. 1c). This is relevant when considering the high antioxidant activity of astaxanthin [19] and its possible involvement in the oxidative stability of the different lipid fractions of shrimp. During shrimp cooking, heat induces denaturation and aggregation of proteins leading to exposure of hydrophobic areas, which allows for new interactions between proteins and other hydrophobic components [3]. The formation of a large number of interactions between the hydrophobic areas of the proteins and free astaxanthin could explain the increased retention of astaxanthin in cooked shrimp.

No significant changes were observed in most of the fatty acids from the lipid fraction by effect of cooking (Table 4). However, a slight declining trend was observed in EPA and DHA when shrimps were boiled in brine with high concentration of salt and/or prolonged cooking time (Table 4). This is in agreement with the results of Souza and Bragagnolo [20], who found slight decreases in EPA and DHA in boiled shrimp after cooking in brine (30% w/w for 10 min). The slight declining trend observed in EPA and DHA could be attributed to greater structural damage in cell membranes and muscle tissue caused by the severe boiling conditions, which together with high salt concentrations, may have contributed to EPA and DHA oxidation as well. In this sense, Niamnuy et al. [17] found significant changes in the microstructure of shrimp muscle after cooking depending on the boiling conditions employed. An undesirable effect arising from the oxidation of fatty acids in the shrimp is the

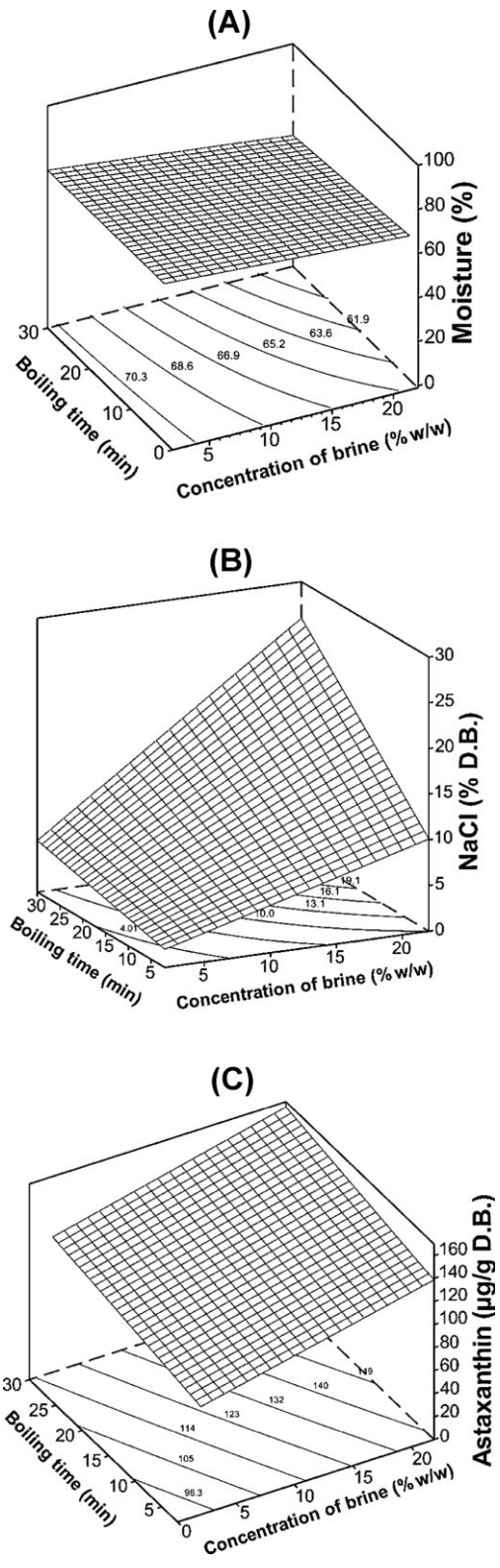

Figure 1. Effect of main cooking conditions on moisture (A), NaCl (B), and astaxanthin content (C) in boiled shrimp.

Table 4. Effect of different boiling conditions on fatty acids profile in shrimp after cooking and sun drying

Concentration of NaCl in brine (% w/w)	Boiling time (min)	SFA	MUFA	PUFA	20:5 n3 (EPA)	22:6 n3 (DHA)
Raw shrimp						
0	0	27.3 ± 0.0 h	29.2 ± 0.1 a	22.4 ± 0.1abcd	9.8 ± 0.0 abcd	7.1 ± 0.0 ab
Boiled shrimp						
5	5	30.0 ± 2.3 fg	22.5 ± 1.8 cd	23.1 ± 1.4 ab	10.2 ± 0.4 ab	7.1 ± 0.1 abc
5	25	29.2 ± 2.4 gh	22.0 ± 2.3 d	21.3 ± 1.7 bcdef	9.0 ± 0.5 bcdef	6.4 ± 0.3 abcde
20	5	31.8 ± 0.2 bcdef	25.9 ± 0.6 b	20.6 ± 0.2 bcdef	8.7 ± 0.0 def	6.3 ± 0.2 cdef
20	25	31.2 ± 0.1 def	24.7 ± 0.2 b	22.4 ± 0.0 abcd	9.3 ± 0.0 abcdef	6.8 ± 0.0 abcd
12.5	15	31.1 ± 0.5 efg	25.4 ± 0.4 b	24.4 ± 0.0 a	10.5 ± 0.0 a	7.1 ± 0.1 a
Boiled shrimp after 24 h of sun drying						
5	5	31.7 ± 0.8 bcdef	26.5 ± 1.2 b	22.1 ± 1.3 abcde	9.9 ± 0.2 abcd	6.4 ± 0.6 abcde
5	25	33.5 ± 0.0 ab	25.6 ± 0.2 b	22.9 ± 0.6 abc	10.0 ± 0.1 abc	6.5 ± 0.0 abcde
20	5	34.0 ± 0.5 a	26.2 ± 0.4 b	19.8 ± 0.7 ef	8.3 ± 0.2 f	5.8 ± 0.3 ef
20	25	33.3 ± 0.5 ab	24.8 ± 0.2 b	22.2 ± 0.1 abcde	9.3 ± 0.2 abcdef	6.3 ± 0.2 abcde
12.5	15	31.2 ± 0.1 cdef	24.3 ± 0.0 bc	21.2 ± 3.0 bcdef	9.1 ± 2.1 bcdef	6.4 ± 0.7 abcde
Boiled shrimp after 40 h of sun drying						
5	5	33.2 ± 0.4 abc	25.2 ± 0.9 b	20.9 ± 1.3 bcdef	8.8 ± 0.5 cdef	6.3 ± 0.5 bcde
5	25	32.6 ± 0.5 abcde	25.7 ± 0.7 b	20.3 ± 0.8 cdef	8.6 ± 0.4 f	5.9 ± 0.6 ef
20	5	33.4 ± 0.2 ab	26.3 ± 1.1 b	19.9 ± 1.5 def	8.6 ± 0.4 ef	5.9 ± 0.5 ef
20	25	32.6 ± 0.1 abcde	25.3 ± 0.3 b	20.8 ± 1.4 bcdef	8.8 ± 0.5 cdef	6.2 ± 0.3 def
12.5	15	33.1 ± 0.9 abcd	25.7 ± 2.0 b	19.5 ± 0.3 f	8.9 ± 0.0 cdef	5.5 ± 0.6 f

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
Fatty acids are expressed as % of total fatty acids.
Each entry represents the means ± SD of three replicates. Different superscripts in each column indicate statistical difference ($p < 0.05$).

formation of compounds such as hexanal, which can be formed from the n-6 fatty acids and have a negative impact on the flavor of the final product [20].

Several COPs commonly present in foods were found in raw shrimp samples (Table 4). Main COPs found were 7α-HC, 7β-HC, α-CE, β-CE, 7-KC, and 25-HC. This profile is consistent with reports by other authors [21]. The occurrence of COPs in raw shrimp could be attributed to the oxidation of cholesterol by auto-oxidation and enzymatic mechanisms. Considering the above, the temperature at which shrimp are handled and the time elapsed from capture to marketing thus becomes essential [21].

No significant changes were observed in the content of different COPs between raw and cooked shrimp (Table 5). Likewise, the cooking conditions did not show influence on the COPs concentration in cooked shrimp (Table 2). The temperature reached during sample boiling ranged from 100 to 104°C. Cholesterol alone is stable at 100°C, but some COPs begin to form when stored with polyunsaturated lipids for long periods of time [7]. The lipid fraction in shrimp had an elevated content of PUFA (Table 4), which could be susceptible to oxidation and thus favor cholesterol co-oxidation during boiling. However, apparently, the slight oxidation of EPA and DHA observed was not enough to generate a substantial amount of COPs in the cooked shrimps. Moreover, it might be also possible that, during boiling, part of the COPs also decomposed and/or reacted with other molecules (such as amino compounds), generating compounds that could not be detected under the analytical conditions used [22]. In this regard, the decrease of EPA and DHA could be better markers of the oxidation in the lipid fraction that COP formation during cooking shrimp.

3.2 Sun drying

Direct exposure to the sun is a traditional technique used in many countries to dry cooked shrimp. Environmental conditions such as the incident solar energy, temperature and relative humidity are important factors during sun drying of foods and vary depending on the geographic location and the season. In the city of Veracruz, it has been estimated that the daily average horizontal amount of sunshine is 4.7 kWh/m^2 day, which is higher than the world average (3.8 kWh/m^2 day) [23]. During the 5 days of sun drying, the ambient temperature ranged from 25 to 37°C and the relative humidity varied from 57 to 88%. The different boiling conditions evaluated in this research did not affect the final moisture content in the dried salted shrimp (Table 1). Moisture content reached in the samples after 24 and 40 h of sun drying was 17.6 ± 3.6 and 12.6 ± 0.8%, respectively.

Table 5. Effect of different boiling conditions on individual and total COPs (μg/g lipids) in shrimp after cooking and sun drying

Concentration of NaCl in brine (% w/w)	Boiling time (min)	7α-HC	7β-HC	β-CE	α-CE	CT	25 HC	7-KC	Total COPs
Raw shrimp									
0	0	110.8 ± 23.0 ef	13.9 ± 19.6 e	16.3 ± 4.6 d	11.4 ± 2.4 f	n.d.	6.2 ± 8.8 de	6.0 ± 8.5 g	164.6 ± 57.7 d
Boiled shrimp									
5	5	75.2 ± 37.1 f	16.8 ± 3.6 e	36.3 ± 12.4 d	11.5 ± 10.4 f	n.d.	9.0 ± 5.6 de	24.5 ± 8.1 g	173.2 ± 77.2 d
5	25	73.5 ± 20.8 f	14.1 ± 7.0 e	33.1 ± 7.0 d	12.4 ± 4.3 f	n.d.	12.6 ± 9.5 bcde	25.3 ± 16.1 g	171.1 ± 64.7 d
20	5	174.9 ± 100.0 cde	26.9 ± 15.8 e	60.2 ± 25.0 d	27.9 ± 10.0 ef	n.d.	14.4 ± 8.8 bcde	41.8 ± 18.9 g	346.1 ± 178.5 d
20	25	126.5 ± 51.7 def	20.3 ± 6.7 e	41.8 ± 11.1 d	17.6 ± 5.0 f	n.d.	9.5 ± 8.1 cde	22.0 ± 9.9 g	237.9 ± 92.6 d
12.5	15	94.9 ± 47.4 ef	21.7 ± 16.5 e	48.2 ± 24.1 d	18.3 ± 10.0 f	n.d.	9.5 ± 7.1 cde	27.5 ± 13.2 g	220.1 ± 118.3 d
Boiled shrimp after 24 h of sun drying									
5	5	258.1 ± 4.1 abc	143.1 ± 6.8 d	126.0 ± 11.2 c	52.6 ± 0.9 cdef	6.9 ± 1.5 b	23.6 ± 2.7 bcde	110.2 ± 20.6 f	720.4 ± 14.3 c
5	25	293.9 ± 28.9 ab	237.5 ± 10.5 ab	189.3 ± 9.7 ab	73.8 ± 6.9 abcd	8.3 ± 0.3 b	58.0 ± 35.8 a	232.2 ± 35.8 bc	1093.1 ± 114.1 ab
20	5	270.9 ± 11.1 ab	243.9 ± 47.7 a	221.6 ± 46.6 a	108.3 ± 68.5 a	11.7 ± 3.6 ab	28.2 ± 39.9abcde	256.0 ± 48.4 b	1140.6 ± 146.9 a
20	25	223.4 ± 48.2 abc	138.2 ± 3.5 d	134.8 ± 16.1 c	49.5 ± 3.1 def	13.8 ± 6.8 ab	27.1 ± 4.2 abcde	133.3 ± 23.4 ef	720.3 ± 11.8 c
12.5	15	226.1 ± 33.6 abc	172.9 ± 39.7 cd	192.9 ± 18.1 ab	60.6 ± 2.4 bcde	4.2 ± 5.9 b	0.0 ± 0.0 e	316.0 ± 32.0 a	972.7 ± 0.5 ab
Boiled shrimp after 40 h of sun drying									
5	5	315.2 ± 58.2 a	219.2 ± 47.7 abc	190.9 ± 31.1 ab	87.4 ± 18.0 abcd	44.6 ± 54.4 a	42.6 ± 13.9 ab	202.8 ± 37.8 bcd	1102.6 ± 261.1 ab
5	25	268.5 ± 9.3 abc	171.1 ± 33.0 cd	169.9 ± 12.3 bc	74.6 ± 14.8 abcd	7.4 ± 1.0 b	41.5 ± 2.2 abc	176.6 ± 38.3 cde	909.7 ± 60.8 abc
20	5	265.2 ± 20.8 abc	178.3 ± 6.6 cd	172.8 ± 4.5 abc	73.2 ± 6.0 abcd	10.6 ± 1.0 ab	34.8 ± 9.8 abcd	148.6 ± 3.4 def	883.6 ± 38.9 bc
20	25	264.0 ± 8.5 abc	181.9 ± 34.0 bcd	209.7 ± 27.5 ab	94.5 ± 10.7 ab	13.6 ± 0.2 ab	34.8 ± 3.3 abcd	184.6 ± 34.2 cde	983.2 ± 90.5 ab
12.5	15	208.7 ± 74.3 bcd	173.2 ± 36.3 cd	192.4 ± 49.1 ab	92.6 ± 17.5 abc	13.9 ± 3.4 ab	19.6 ± 1.3 bcde	173.9 ± 38.4 cde	874.2 ± 178.4 bc

COPs, cholesterol oxidation products; 7α-HC, 7α-hydroxycholesterol; 7β-HC, 7β-hydroxycholesterol; α-CE, 5,6α-epoxycholesterol; β-CE, 5,6β-epoxycholesterol; 7-KC, 7-ketocholesterol; 20-HC, 20-hydroxycholesterol; 25-HC, 25-hydroxycholesterol; CT, cholestanetriol; n.d. no detected.
Each entry represents the means ± SD of three replicates. Different superscripts in each column indicate statistical difference ($p < 0.05$).

Astaxanthin content in cooked shrimp decreased considerably during solar drying (Table 1). The decline in astaxanthin content in shrimp mainly affects product color, which is a major quality attribute in dried shrimp [1]; therefore, a decrease in the content of astaxanthin from overexposure of shrimp to sunlight is not convenient. Furthermore, considering the high antioxidant activity of astaxanthin [19], degradation of this carotenoid could also have a negative effect on the oxidative stability of lipids. There was no evidence of any effect of brine concentration and boiling time on astaxanthin content reached in dried shrimp after 24 and 40 h of sun drying (Tables 1 and 2). The amount of astaxanthin degraded after 40 h of sun drying was 77.6%, reaching 28.7 ± 6.5 μg/g (d.b.) This astaxanthin content was much lower than that obtained in a jet spouted bed drier at 80, 100, and 120°C [17]. These authors noted that if the drying temperature decreased, the content of astaxanthin retained was smaller, indicating greater carotenoid degradation. It was concluded that lower temperatures required longer drying times, which promoted 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 [24]. This would explain the rather high degradation rate of this carotenoid observed during sun drying of shrimp.

Both the thermal and photochemical degradation of astaxanthin have been studied in several model systems [24, 25]. Like in other carotenoids, these processes generally produce isomers (*trans*, mono-*cis* and di-*cis*), oxidation products (apo-carotenoids and epoxides), volatile compounds (such as β-ionone) and non-volatile chain breaking compounds [26]. Although formation of several compounds derived from the degradation of carotenoids could involve only a reduction in the antioxidant capacity, Bragadóttir et al. [27] have suggested that these oxidation products can act as pro-oxidants affecting other molecules.

Significant decreases in the PUFA content have been reported during sun drying of fish [28]. However, in this study the PUFA content in shrimp was not affected by the cooking conditions and did not change during sun drying (Tables 2 and 4). The lipid fraction of shrimp is mainly formed by 72–74% phospholipids, and 16% TAGs [29], and most PUFA are esterified to phospholipids rather than to TAGs. It has been established that marine phospholipids are more resistant towards oxidation than bulk fish oil (mostly TAGs) from the same source [30]. This could explain the non-significant changes observed in PUFA during sun drying.

The concentration of each COP identified in boiled shrimp increased substantially after 24 h of sun drying (Table 5). Direct exposure to UV and VIS light during sun drying of shrimp could be a major variable that caused oxidation of cholesterol in the samples by photo-oxidation [31]. Several reports 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 [31–34]. Other factors, such as temperature reached during drying, the presence of oxygen and pro-oxidants, could have also contributed to cholesterol oxidation in shrimp.

The mechanisms of cholesterol oxidation under different conditions have been addressed by several researchers [32, 33]. 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 [31, 32]. Moreover, singlet oxygen formed by triplet sensitizer-ground state oxygen interaction can react with cholesterol by a non-radical mechanism, producing mainly 5α-OOH. The 5α-OOH tends to convert rapidly into 7α-OOH, which later isomerizes to 7β-OOH [31–33]. 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 can be converted to 7-KC by dehydrogenation [35]. During sun drying of shrimp, a greater formation of 7α-HC was observed as compared to 7-KC (Table 5), which suggests that equilibrium of the dysmutation reaction of hydroperoxides favor their conversion into the corresponding hydroxyl derivatives. As observed for hydroperoxides, 7α-HC is commonly found at lower concentrations levels than its corresponding epimer (7β-HC), as the α is less favored from a thermodynamic standpoint [33]. However, the presence of photosensitizers may provide an environment for promoting photo-oxidation, and thus the formation of 7α-HC is favored [32]. In our study, the production of 7α-HC was similar to 7β-HC, possibly due to an increase in the formation of 7α-OOH through a photo-oxidative pathway.

Another path of cholesterol oxidation occurs when a hydroperoxyl radical and cholesterol interact to produce α-CE and β-CE through a mechanism similar to that observed in MUFA oxidation [33]. 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 COP found in foods; however, its presence is always less than 7-HC (α and β), 7-KC, and CE (α and β) [7].

The increase in concentration of 7β-HC, β-CE, 7-KC, and total COPs observed in shrimp after 24 h of sun drying was affected by the interaction between brine concentration and boiling time (Tables 2 and 5). Table 3 shows the coefficients of the first-order models calculated to estimate the 7β-HC, β-CE, 7-KC, and total COPs content in dried salted shrimp after 24 h of sun drying. In this regard, the lowest content of total COPs (Table 5) were observed in shrimp boiled in brine with low salt concentration and short boiling time, and also in samples cooked in brine with high salt concentration and prolonged boiling time (Fig. 2). The inhibition in COPs formation into boiled shrimp under mild conditions (5% w/w of brine and 5 min of cooking time) during sun drying could be due to low susceptibility to oxidation of cholesterol located inside of non-damaged cell membranes. During boiling, changes in tissue structure such as destruction of cell membranes, shrinkage of muscle fiber, aggregation of sarcoplasmic protein and shrinkage and solubilization of stromal proteins also occur. The extent of alterations in the microstructure of shrimp depends on brine concentration and boiling time [17]. Conversely, low COPs formation was also noted in cooked shrimp under more severe conditions (20% w/w of brine and 25 min of cooking time). As already mentioned, this might be due to partial decomposition of COPs and/or reaction with other molecules (such as proteins), thus generating different compounds [22]. Although shrimp cooking under this condition promoted structural damage in shrimp muscle and increased the exposure of cholesterol to oxidation, it is also remarkable the elevated retention of astaxanthin observed in shrimp after boiling (Table 1). Astaxanthin is a carotenoid that is characterized by its high capacity to scavenge free radicals and to quench singlet oxygen [19]. This could explain the low cholesterol oxidation into cooked shrimp during the early hours of sun drying.

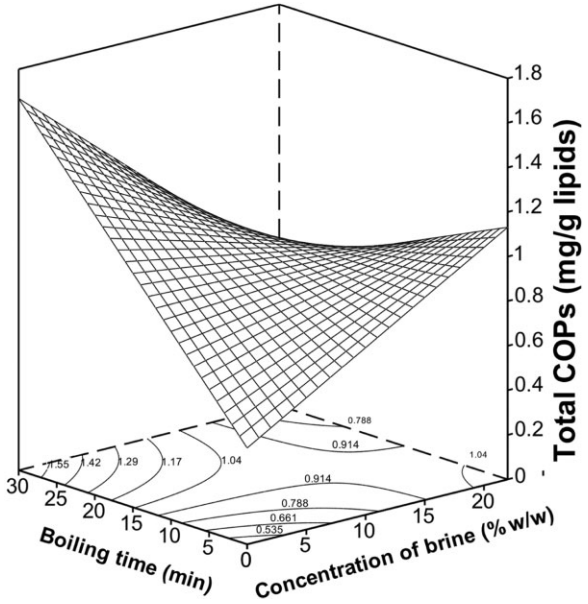

Figure 2. COPs formation in shrimp boiled under different cooking conditions after 24 h of sun drying.

Shrimps dried by exposure to direct sunlight for 24 h, with the lowest levels of total COPs previously observed (boiled shrimp in brine at 5% w/w for 5 min and 20% w/w for 25 min), exhibited a significant increase in the total content of COPs after 40 h of solar drying (Table 5). On the other hand, the dried shrimps, with the highest total COPs content observed after 24 h of solar drying (boiled shrimp in brine at 5% w/w for 25 min, 20% w/w for 5 min and 12.5% w/w for 15 min) showed no significant changes in their high content of COPs after 40 h of solar drying (Table 5). This is technologically relevant if dried salted shrimp with low levels of COPs is desired. Therefore, we recommend boiling the product in brine at 5% for 5 min and avoid overexposing to direct sunlight for no more than 24 h during solar drying. Although cooking of shrimp under severe conditions (20% w/w of brine and 25 min of cooking time) displayed a reduced formation of COPs after 24 h of solar drying, it is important to note that significant amounts of other undesirable compounds, such as malondialdehyde, epoxy derivatives, oxidized cyclic monomers, oxidized dimers, and hexanal, may be formed under these cooking conditions and these compounds could be further increased during solar drying. Furthermore, the formation of compounds such as hexanal [20] together with color fading due to astaxanthin reduction (Table 1), could have a significant impact on the sensory attributes of this product and thus affect its commercial trading.

3.3 Storage

Dried salted shrimp is a product with an elevated salt concentration and low water content, which favors a long shelf-life depending on the process and storage conditions [36]. Table 6 shows the changes in astaxanthin and COPs content, as well as fatty acids profile in dried shrimp after 75 and 150 days of storage under either daylight conditions and in the dark. Astaxanthin concentration in dried shrimp decreased significantly after 75 days of storage. However, no significant difference between the samples stored with or without light protection was observed. Similarly, no significant difference was observed in astaxanthin content after 150 days of storage (Table 6). Astaxanthin is sensitive to photodegradation [24]; nevertheless, other variables such as temperature and oxygen availability have proved to exert greater influence on the degradation of this carotenoid during storage [3].

Significant decreases in the PUFA content were observed during storage (Table 6), especially EPA and DHA. This is in agreement with the results of Cardenia et al. [34], where they found 33 and 19% decrease in DHA and total PUFA contents, respectively, in sardine fillet stored under light exposure. 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 [7]. 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.

The concentration of each COP identified in dried shrimp increased significantly during the storage (Table 6). The photo-oxidation of cholesterol during storage of different meat products under commercial conditions has demonstrated to be an important mechanism of COPs formation [31, 34]. In the present study, samples stored 75 and 150 days under darkness showed COPs content similar to samples unprotected from incident daylight (Table 6). This suggests that photo-oxidation may not be the main mechanism responsible for the increase of COPs in dried shrimp during storage. In this regard, it is important to consider that stored samples were exposed to sun drying, where most of the cholesterol from the superficial tissue had been photo-oxidized. Therefore, the increase in concentration of COPs during storage could be attributed to the oxidation of cholesterol from internal tissues through other alternative mechanisms, such as autoxidation. After 75 days of storage, no effect of boiling time on the COPs formation was observed. However, after 150 days shrimp boiled for 25 min showed higher total COPs content than the samples boiled by 5 min (Table 6). The total COPs content in dried salted shrimp during storage ranged from 0.9 to 2.4 mg/g of lipids, which corresponds to 8.7–23.4 mg/100 g dried shrimp (d.b.). This is consistent with the data reported by Soto-Rodríguez et al. [9], who found concentrations of COPs from 13.0 to 25.4 mg/100 g d.b. in commercial samples of dried shrimp. On the other hand, dried salted shrimp from different seasons and regions in Brazil showed a total content of COPs from 1.4 to 11.4 mg/100 g of dried shrimp (d.b.) [8]. 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 key variable in the formation and accumulation of COPs in dried shrimp, as it is shown in Table 6. 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 still required to better ascertain the toxicity levels of particular COPs [6]. However, according to the threshold of toxicological concern (TTC) for unclassified compounds (0.15 μg per person per day) [37], the consumption of dried salted shrimp with COPs levels as those found in the present study could be considered as a health hazard.

4 Conclusions

The effects of shrimp cooking conditions on oxidative processes experienced by the main components of the lipid fraction from dried salted shrimp during processing and storage, were investigated. Boiling conditions did not affect

Table 6. Effect of different boiling conditions on moisture content (g/100 g), NaCl (g/100 g d.b.), astaxanthin (μg/g d.b.), individual and total COPs (μg/g lipids), and fatty acids profile in dried salted shrimp during storage

			75 Storage day				150 Storage day			
	0 Storge day		Daylight		Dark		Daylight		Dark	
Conc. of NaCl in brine (% w/w)	5	5	5	5	5	5	5	5	5	5
Boiling time (min)	5	25	5	25	5	25	5	25	5	25
Moisture	12.1 ± 0.5 cd	13.0 ± 0.6 bc	12.0 ± 0.1 cd	12.5 ± 0.1 cd	11.6 ± 0.2 d	11.9 ± 0.1 d	13.9 ± 0.1 b	15.8 ± 1.1 a	14.9 ± 0.5 a	14.9 ± 0.0 a
Astaxanthin	24.1 ± 1.1 b	31.2 ± 7.8 a	4.4 ± 3.0 c	4.9 ± 0.3 c	7.3 ± 2.9 c	9.5 ± 0.3 c	6.1 ± 0.3 c	6.3 ± 1.2 c	6.2 ± 0.4 c	5.5 ± 0.3 c
COPs										
7α-HC	315.2 ± 58.2 b	268.5 ± 9.3 bc	251.1 ± 18.2 bcd	211.2 ± 11.5 cde	151.9 ± 54.7 e	200.1 ± 74.6 cde	177.7 ± 10.7 de	275.0 ± 19.7 bc	241.9 ± 13.3 bcd	402.6 ± 43.4 a
7β-HC	219.2 ± 47.7 de	171.1 ± 33.0 e	375.3 ± 89.7 ab	305.6 ± 69.7 bcd	285.1 ± 79.5 bcd	229.8 ± 60.6 de	247.5 ± 7.1 cde	354.3 ± 54.4 abc	317.1 ± 6.1 bcd	462.0 ± 42.5 a
β-CE	190.9 ± 31.1 ef	169.9 ± 12.3 f	373.3 ± 70.1 bcd	360.4 ± 43.8 bcd	345.1 ± 101.4 bcd	260.5 ± 63.4 def	300.8 ± 5.1 cde	434.9 ± 73.0 ab	392.0 ± 11.4 bc	544.5 ± 55.6 a
α-CE	87.4 ± 18.0 bcd	74.6 ± 14.8 d	120.0 ± 24.5 ab	100.2 ± 11.7 bcd	98.0 ± 25.4 bcd	76.4 ± 19.7 d	82.8 ± 7.0 cd	118.0 ± 22.5 abc	104.0 ± 1.7 bcd	144.8 ± 15.0 a
CT	44.6 ± 54.4 cd	7.4 ± 1.0 d	53.1 ± 18.6 bc	58.8 ± 2.4 bc	48.3 ± 0.5 c	49.0 ± 14.7 c	60.4 ± 0.9 c	89.2 ± 0.9 ab	69.8 ± 5.6 bc	114.4 ± 9.9 a
25 HC	42.6 ± 13.9 a	41.5 ± 2.2 ab	37.2 ± 7.3 abc	32.2 ± 0.3 abcd	25.1 ± 6.2 cd	24.4 ± 10.8 cd	19.1 ± 0.7 d	29.1 ± 3.8 bcd	27.3 ± 2.6 cd	34.5 ± 2.8 abc
7-KC	202.8 ± 37.8 e	176.6 ± 38.3 e	490.6 ± 164.0abc	440.2 ± 43.8 bcd	397.6 ± 125.0 bcd	317.6 ± 78.7 de	336.3 ± 6.4 cde	525.0 ± 117.1 ab	425.3 ± 8.3 bcd	649.1 ± 46.9 a
Total COPs	1102.6 ± 261.1de	909.7 ± 60.8 e	1700.7 ± 392.3bc	1508.6 ± 178.3 bcd	1351.1 ± 391.6 bcde	1157.8 ± 322.5 de	1224.6 ± 11.6 cde	1825.6 ± 291.5 b	1577.4 ± 32.8bcd	2351.9 ± 216.0 a
SFA	33.2 ± 0.4 ab	32.6 ± 0.5 b	32.4 ± 0.8 b	34.2 ± 2.3 ab	32.4 ± 0.6 b	33.6 ± 0.7 ab	32.9 ± 1.3 ab	33.7 ± 0.3 ab	33.1 ± 1.1 ab	34.8 ± 1.1 a
MUFA	25.2 ± 0.9 b	25.7 ± 0.7 ab	25.4 ± 0.6 ab	26.0 ± 1.3 ab	24.6 ± 2.2 b	26.4 ± 1.6 ab	25.6 ± 0.5 ab	27.7 ± 1.2 a	25.3 ± 0.0 b	26.5 ± 0.3 ab
PUFA	20.9 ± 1.3 a	20.3 ± 0.8 ab	18.0 ± 1.9 bc	17.2 ± 0.2 c	17.2 ± 1.0 c	17.8 ± 0.1 c	17.4 ± 2.0 c	16.0 ± 0.2 c	17.6 ± 1.8 c	16.7 ± 0.3 c
20:5 n3 (EPA)	8.8 ± 0.5 a	8.6 ± 0.4 ab	7.8 ± 0.9 abc	7.4 ± 0.4 bc	7.2 ± 0.3 c	7.6 ± 0.6 abc	7.6 ± 1.0 abc	7.1 ± 0.2 c	7.5 ± 1.2 bc	7.4 ± 0.1 bc
22:6 n3 (DHA)	6.3 ± 0.5 a	5.9 ± 0.6 ab	5.4 ± 0.7 abc	5.1 ± 0.4 bc	5.1 ± 0.1 bc	5.2 ± 0.2 bc	5.3 ± 0.8 bc	4.8 ± 0.1 c	5.4 ± 0.6 abc	4.8 ± 0.3 c

COPs, cholesterol oxidation products; 7α-HC, 7α-hydroxycholesterol; 7β-HC, 7β-hydroxycholesterol; α-CE, 5,6α-epoxycholesterol; β-CE, 5,6β-epoxycholesterol; 7-KC, 7-ketocholesterol; 20-HC, 20-hydroxycholesterol; 25-HC, 25-hydroxycholesterol; CT, cholestanetriol; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Fatty acids are expressed as % of total fatty acids.

Each entry represents the means ± SD of three replicates. Different superscripts in each row indicate statistical difference ($p < 0.05$).

the content of COPs in shrimps after cooking. However, increasing brine concentration and boiling time promoted higher astaxanthin retention in cooked shrimp. During the first 24 h of sun drying, COPs formation in dried salted shrimp was influenced by the interaction between brine concentration and boiling time. In this regard, the lowest COPs levels were observed in samples boiled at low concentration brine and short cooking time, as well as those boiled in brine with high salt concentration and longer time. This is technologically relevant if preparation of dried salted shrimp with low levels of COPs is desired. Most astaxanthin (78%) present in cooked shrimp was degraded during solar drying. During storage, PUFA decreased, while a marked astaxanthin degradation and concomitant COPs formation in dried salted shrimp were observed. However, boiling conditions as well as storage of dried salted shrimp in the dark did not inhibit these changes. To obtain dried salted shrimp with a better oxidative stability, alternative processing, and technological strategies should be used for the production of such widely consumed seafood product, so as to assure a better preservation of its overall nutritional profile to consumers.

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