

Characterization of Lipids and Their Oxidation Products in Baked or Fried Breaded Shrimp Products

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Abstract: Baking and frying are two common food preparation methods, but there is limited knowledge about the lipid oxidation events that occur in food products prepared in these ways. Two commercially available breaded shrimp products were examined using gas chromatography, for changes in cholesterol, phytosterols, and fatty acids as a result of baking and deep frying after separation into breading and shrimp components. Additionally, changes in oil quality factors were determined for the cooking oils used to prepare the fried products. Sterol concentrations changed in both shrimp products due to degradation. Oxidation products of cholesterol and fatty acids were found in the shrimp portions of both the baked and fried samples as well as the oils used for frying using gas chromatography in tandem with mass spectroscopy. Phytosterol oxides were detected in the frying oils, but not in the baked or fried shrimp products. The frying oils showed some oxidative losses of phytosterols. This work demonstrated that measurable lipid oxidation occurred, even over the short time periods needed to prepare these products. This may have implications for human health effects.

Keywords: Shrimp, frying, oxidation, fatty acids, sterols.

Thermal processing of foods typically induces changes that make products more appealing for consumption and improves nutrient availability and food safety. Baking or roasting involves exposure of products to high temperatures in air, often on metal surfaces. Deep frying relies on the use of an oil medium to transfer heat to the food product. This process also involves exposure of the food and its lipid components to air and metal surfaces from processing equipment. The oxidation of fatty acids and sterols is a free radical process [1]. The exposure of these compounds to conditions of high heat, air and light and often in the presence of metal ions as catalysts can result in the breakdown of unsaturated carbon-carbon bonds found in these compounds, accelerating lipid oxidation.

The oxides of cholesterol produced in food products have been studied and discussed for almost two decades and have been reviewed [1, 2]. Shrimp in particular have been the subject of recent studies on cholesterol oxidation products [3, 4]. Investigations into the types of products formed and their effects on biological systems have shown that cholesterol oxides (COPS) are cytotoxic and mutagenic and have been implicated in the onset of arteriosclerosis and coronary heart disease in humans [5, 6]. The structural similarity of plant sterols or phytosterols to cholesterol (Fig. 1) would indicate that similar types of oxidation products could be formed when phytosterol containing foods are subjected to the oxidizing conditions of many food processing operations.

In the past, the main focus was on the oxidation of cholesterol from lard, tallow and meat containing products [7]. To date, there have been fewer studies published on phytosterol oxidation products (POPS). Work done with model systems and synthesized compounds showed that POPS are produced, but few studies are available on total food systems [8-10]. The few studies available focused on potato products cooked in vegetable oils [11-13]. In those studies, oxidation products of campesterol and sitosterol were isolated from the fried potatoes at the low ppm level and the concentrations increased with storage time. More recent research into the types of plant sterol oxidation products produced in rapeseed oils and sterol enriched rapeseed oils used for frying found that the POPS produced were mainly 7-hydroxy, 5,6-epoxy and 7-keto compounds that increased with time of frying [14, 15].

This study examined the lipid changes in a system which combined both a cholesterol source (shrimp) with phytosterol sources (bread coating and vegetable oil). Cooking conditions included heating with (frying) and without (baking) added oil and were designed to replicate the typical styles of preparation used by consumers of frozen, breaded seafood products. Changes in the quality of the oil used for frying before and after cooking the shrimp were monitored, as were the fatty acids in the oil and the shrimp components. The fate of sterols present in the breading, shrimp, and oil was also examined and the oxides of the sterols produced were investigated.

MATERIALS AND METHODS

Samples and Reagents

Two different lots of two different brands of frozen breaded shrimp products were purchased from a local gro-

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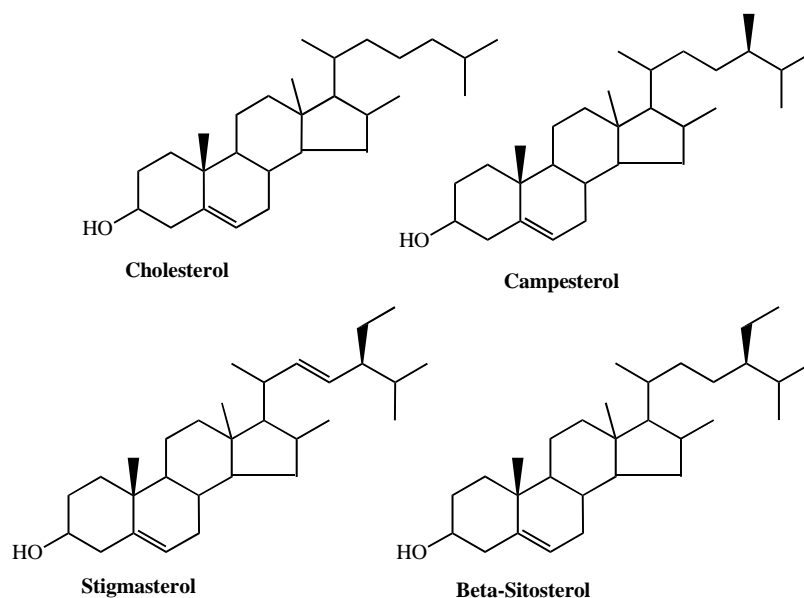


Fig. (1). Structures of some common sterols

cery store and designated as Product 1 and Product 2. Each sample was tested in the uncooked state to establish control conditions and then tested after cooking by both baking and frying. All reagents and chemicals used in the analysis were purchased from Thermo-Fisher (Fair Lawn, NJ) unless otherwise noted.

Sample Preparation

Products were packaged frozen and advertised to be prepared by either baking or deep frying. For analysis in the uncooked state, the entire contents of the packages (227 to 567 grams) of the products were allowed to thaw at room temperature. Shrimp and the breading components were separated by gloved hands and homogenized separately by grinding in a small food processor (Cuisinart MiniPrep, East Windsor, NJ) at low speed. Both lots of each sample were homogenized together to produce a single homogenate for each product. The homogenates of the separated shrimp and breading were packed in Zip-lock® (S. C. Johnson, Racine, WI) plastic bags, flushed with nitrogen and stored in a freezer at -20°C until analyzed within a period of two weeks. The moisture of each homogenate was determined gravimetrically after drying in a vacuum oven (VWR Scientific, West Chester, PA) at 100°C for 5 hr according to AOAC Method 925.09 [16].

One set of samples was prepared by baking. The cooking instructions provided on the retail packages for each product were very similar (10 to 12 min for Product 1 and 9 to 11 min for Product 2), so the same cooking procedure was used for each product. Product pieces were spread on an aluminum baking pan while frozen. The pan was placed in a household oven that had been preheated to 218°C (425°F). The temperature was checked with a thermocouple (Omega Model HH-22, Omega Engineering, Inc., Stamford, CT). The product was cooked in the oven for 10 min, then removed and cooled to room temperature. The product was then separated by hand and the breading and shrimp portions were homogenized and stored as described for the raw portions.

Additional portions of the products were prepared by frying according to the manufacturer's instructions. Soybean oil (Proctor and Gamble, Cincinnati, OH) was added to a cast iron frying pan to a depth of 20 mm. The oil was heated to 180°C (350°F) on an electric burner. Oil temperature was determined using a thermocouple before the product was added. The products in the frozen state were added to the hot oil. The pieces were held in the hot oil for 4 min and turned once during the heating cycle. The pieces were cooled to room temperature and separated by hand into the shrimp and breading components. Each lot was then homogenized together so that a single homogenate was used for the analysis of each product. Samples of the cooking oil before and after the heat treatment were also collected for analysis. Fresh oil was used for frying each replicate. Each sample of oil was analyzed in triplicate for the parameters described below. The ratio of product to oil was 112 g to 180 mL.

Oil Quality

Changes in the quality of the oil used for frying the products were examined. Using AOCS methods [17], the peroxide values (PV) (AOCS Cd 8-53), free fatty acids (FFA) (Ca 5a-40) and oxidative stability indices (OSI) (Cd 12b-92) were determined for the oil before and after frying the products. Oil color was measured using a Lovibond Model E Tintometer (Lovibond Ltd, Salisbury, UK).

Tocopherols in Oils

The oil used for frying the products was analyzed for individual and total tocopherols before and after the cooking process. The weighed amounts of the oil samples were diluted with hexane and injected on an HPLC (Agilent Series 1100, Agilent Technologies, Santa Clara, CA) according to the method of Hashim and others [18]. The separation was made on a Luna Silica column (250 mm x 4.6 mm, 5 micron) obtained from Phenomenex (Torrance, CA). The detection was by UV at a wavelength of 294 nm. The mobile phase was 1% isopropanol in hexane at a flow rate of 1.2 mL per min. The individual tocopherols present were determined by

comparison to standard curves prepared by diluting authentic standards of alpha, beta, gamma, and delta tocopherol (Matreya, Pleasant Gap, PA) with hexane. The results were reported in Table 2 as the total of all the tocopherols.

Fatty Acid Contents

The homogenates from the products and the frying oil were analyzed for their fatty acid content after derivatization to fatty acid methyl esters (FAMES) according to the method described by Park and Goins [19]. Briefly, 100 mg of each sample was weighed into a glass screw topped tube and 1 mL of methylene chloride was added to solubilize the lipid. After the addition of 1 mL of 0.5 N sodium hydroxide in methanol, the tubes were heated for 10 min in a water bath at 90° C, cooled to room temperature and 1 mL of 14% boron trifluoride in methanol (Sigma Chemical Corp., St. Louis, MO) was added. The tubes were recapped, vortexed for 30 sec and returned to the water bath for 10 min. The tubes were removed from the heat and allowed to cool to room temperature. One mL of water was added, followed by 1 mL of hexane. The tubes were vortexed and allowed to stand at room temperature for 30 min until layers formed. A portion of the top (organic) layer was transferred into a GC vial containing a few grains of sodium sulfate.

Samples were analyzed for their FAMES content by GC against authentic standards of fatty acid methyl esters (Kel-Fim-Fame-5 mix, Matreya, LLC, Pleasant Gap, PA). The separation of the fatty acid methyl esters was done on a Perkin Elmer Autosampler System GC (Norwalk, CT) equipped with a flame ionization detector (FID). The capillary column used contained a stationary phase composed of 10% cyanopropylphenylpolysiloxane crossbonded to 90% bicyanopropylsiloxane (RTX-2230, 105 m length, 0.25 mm interior diameter, 0.20µm film thickness, Restex, Bellefonte, PA). Temperature programming used an initial temperature of 100° C held for 2 min. The temperature was increased by 10° C/min to 200° C and held for 0.5 min. The temperature was then increased by 4° C/min to a final temperature of 260° C and held for 8.5 min. The carrier gas was helium set to a flow rate of 0.7mL/min with a split ratio of 3:1. The injector and detector temperatures were 230° C and 265° C, respectively. The data collection and integration was done using ChromPerfect software (Justice Innovation, Version 3.52, Palo Alto, CA). All samples were analyzed in triplicate. The fatty acids were identified by comparison of retention times with authentic fatty acid methyl esters obtained from Matreya (Pleasant Gap, PA). The values were normalized to 100% of the total fatty acids as described in AOAC method Ce 1h-05 [17].

Sterols in Breaded Shrimp Components

The homogenates from each product were analyzed in triplicate for cholesterol, campesterol, stigmasterol, and Beta-sitosterol. Sample components were analyzed in the raw, baked, and fried states. The samples were cold-saponified and extracted according to the method of Dutta and Appvelqvist [12]. In brief, 0.5 g samples were weighed into large screw capped glass culture tubes to which 10 mL of 2 N potassium hydroxide (KOH) in ethanol was added. Tubes were flushed with nitrogen and stored in the dark at room temperature for 24 hr. After the addition of 10 mL water, the samples were extracted with 10 mL of methylene

chloride by shaking. The tubes were allowed to stand 20 min until layers formed and the top layer (aqueous) was removed by suction and discarded. The sample extract was washed twice by adding 10 mL of water, vortexing, allowing layers to form and removing the top layer by suction. Finally, the solvent was dried by passing through sodium sulfate. A 1 mL portion of the methylene chloride extract was transferred to a separate tube where the solvent was evaporated by a nitrogen stream. The residue was taken up in 1 mL of hexane containing 50 µg of 5- α -cholestane (Aldrich Chemical Company, St. Louis, MO) as an internal standard. Extracts were derivatized to their trimethylsilyl ethers using Regisil® (Regis Technologies, Morton Grove, IL) for the GC for the analysis of cholesterol, campesterol, stigmasterol, and Beta-sitosterol. A standard mix of cholesterol and plant sterols (Matreya) was derivatized in the same manner and run with the samples.

The separation of the sterol derivatives was done on a Perkin Elmer Autosampler System GC equipped with an FID. A capillary column containing a stationary phase of 50% methyl polysiloxane crossbonded to 50% phenyl polysiloxane (Restek, Rtx-50, 30 m length, 0.32 mm internal diameter, 0.25µm film thickness) was used. The GC was temperature programmed from 100° C (0.2 min hold time) to 290° C at a rate of 10° C/min with a final hold time of 8 min. The injector and detector temperatures were set to 230° C and 305° C respectively. Helium was the carrier gas set at a flow rate of 2.1 mL/min. Data collection and integration was done using ChromPerfect software. All samples were analyzed in triplicate.

GC-MS for Identification of Sterol Oxides in Breaded Shrimp Components

The frying oil was examined for the presence of oxidation products of the sterols present that may have been generated during the heating using GC-MS. Product 1 was chosen to be analyzed in the same way to identify any oxides of cholesterol and the phytosterols that may have been produced in the food itself. Samples were saponified as described in the section above. After removal of the methylene chloride and reconstitution of the dried extracts with hexane, the sterol oxides were concentrated according to the method validated by Johnsson and Dutta [20]. In brief, an amino SPE cartridge (0.5g, Alltech, Deerfield, IL) was preconditioned by rinsing with 5 mL of hexane. The sample in hexane was added and allowed to flow through by gravity. The column was washed to remove esterified compounds by two rinsings of 2:1 (v:v) hexane:methyl t-butyl ether (MTBE). The sterol oxides were eluted by rinsing the cartridge with 5 mL of acetone. Acetone was removed by evaporation with N₂ and the residue was dissolved in 5 mL of hexane and the enrichment was repeated using a fresh SPE cartridge. Acetone was again removed and the samples were taken up in 1 mL hexane containing 50 mcg/mL of 5- α -cholestane and derivatized to trimethylsilyl-ethers using Regisil® as described in the previous section. The derivatives were analyzed using a Autosystem XL GC System (Perkin Elmer) interfaced to a TurboMass Gold mass spectrophotometer using TurboMass software (Version 5.2). The GC was fitted with a DB-5 MS column (5% phenyl polysilphenylene-siloxane, 30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) from J&W (Folsom, CA). The GC was temperature programmed

from 50°C (1 min hold time) to 275°C at a rate of 20°C/min with no hold time and then at 1°C/min to 290°C with a hold time of 5 min for a total run time of 32 min. The injector was set to a temperature of 290°C with a split flow of 50 mL/min. Helium was the carrier gas set at a flow rate of 1.3 mL/min. The mass spectra were recorded at an electron energy of 70 eV and the scan ranged from 100 to 600 m/Hz. The electron impact (EI) source temperature was 180°C with an inlet line temperature of 300°C.

Statistical Analysis

The data were expressed as the mean of three measurements \pm standard deviation. Analysis of variance (ANOVA) followed by means separation using Duncan's multiple range test [21] was performed to determine differences in lipid measurements among treatments.

RESULTS AND DISCUSSION

The two products tested were similar based on their ingredient statements. Both products listed shrimp, wheat flour and partially hydrogenated vegetable oil as the major ingredients. Table 1 compares the proximate composition of the two products taken from the Nutritional Facts panel of the retail containers of the products and from laboratory analyses. The major source of fat in the raw products was the vegetable oil added to the breading component. Shrimp in the raw or canned state contains approximately 1% total fat [22]. Product 2 was seen by the package label to contain a slightly smaller proportion of carbohydrate than Product 1 (23% vs 24%). The appearance of the product was a drier, harder breading coating, but was found to be higher in moisture.

Table 1. Proximate Composition of the Breaded Shrimp Products

Component	Product 1	Product 2
Total Fat (%)*	10.96 \pm 1.26	14.46 \pm 1.27
Saturated Fat (%)*	2.86 \pm 0.32	2.41 \pm 0.19
Protein (%)†	9.89	9.64
Carbohydrate (%)†	24.18	22.89
Moisture (%)*	42.78 \pm 3.25	50.20 \pm 4.17

*as measured in product as packaged.

†from the Nutritional Facts panel on product package.

The nature of the breaded shrimp systems resulted in the need for cooking times of 10 min for the baked samples and 4 min for the fried ones. The oil was analyzed before and after frying to determine if any oxidation had occurred. As the most concentrated source of lipid in the system, the cooking oil would serve as an indicator of lipid oxidation due to the heat treatment. Table 2 lists the lipid components and oil quality values of the oils before and after the frying of products.

Studies on the deterioration of oils used for frying are often conducted over periods of hours or longer [23-25] and have shown that the quality of oils, as defined by parameters such as PV and tocopherol retention, decreased with time of heating and contact with food. For this work, frying times

were only 4 min and oils were only used once; however, significant changes were observed in color, PV, FFA, OSI, tocopherols and sterols (Table 2). For the tocopherols and sterols, the cooked samples differed significantly from the raw material, but were not significantly different between the two products. The Lovibond red and yellow color increased indicating polymerization of fatty acids had occurred in the oil [26]. PV increased almost 10 fold in Product 1 and by a factor of 6 in product 2 after frying compared to the starting value of 0.85 meq/kg of oil. OSI decreased and the FFA values were slightly increased after frying both products as shown in Table 2. PV is a direct measurement of the production of oxides of the unsaturated fatty acid bonds in oil. The production of volatile materials in the oil due to oxidation was reflected by the decrease in the stability index (OSI) of the oils. Water introduced into the oil system by the shrimp portion of the products was also expected to induce lipid oxidation [15]. Oxidation in oil is also known to be induced by heat and metal contact as well as other factors such as light and air [26]. Given the short periods of frying in this work, we suspected that heat and metal contact, in this case, the iron pan, would be the main catalyst of oxidation. Decreases in the phytosterols indicated that phytosterol degradation and possibly oxidation had occurred. A small amount of cholesterol migrated from the products into the cooking oil during frying as evidenced by the increase in cholesterol post frying.

The amounts of the various sterols recovered from the shrimp and breading components of the cooked products are displayed in Figs. (2 and 3). It was found that the two products behaved somewhat different under the cooking conditions. No oil was added to the baked product, only dry heat. To look first at the shrimp components (Fig. 2), the cholesterol in the Product 1 was not different on a dry weight basis between the raw and the baked. The fried product show a loss of nearly half the cholesterol content indicating deterioration into oxides or perhaps by being carried out of the product into the cooking oil. Since cholesterol oxides were seen as discussed below in the cooking oil and some cholesterol was found in the cooking oil after frying, it seems reasonable that both events have occurred. In Product 2, there was a decrease in the cholesterol content of the shrimp after both baking and frying, although the loss on frying was much greater than that occurring during baking.

In the breading portion of the products (Fig. 3), there was significant decrease in the phytosterols in Product 1 after baking. Since no oil was added during this process, it is expected that the decrease here is due to deterioration of the sterols by heat. In Product 2, there was no significant change. Physically, these two products had different appearances before cooking. Product 1 had a softer moister breading, while that of Product 2 was harder. It can be postulated that the harder breading made the sterols less available to deterioration. Based on the reasoning that the surface breading would be expected to be able to absorb oil during cooking, it would be expected that an increase would be seen in the phytosterols in these components after frying. This would be of some health advantage in that these compounds are known to have cholesterol lowering properties [27]. This was only seen in Product 2. In Product 1, the loss of moisture did not translate into an increase in the phytosterol content, but rather less phytosterols indicating degradation.

Table 2. Changes in Components of Soybean Oil Used as Frying Medium for Breaded Shrimp Products

Component	Before Frying	After Frying Product 1	After Frying Product 2
Saturated Fatty Acids [*]	14.97 ± 0.08 ^a	15.32 ± 0.07 ^b	15.08 ± 0.07 ^a
Monoenes [*]	23.50 ± 0.08 ^a	23.43 ± 0.03 ^a	23.74 ± 0.18 ^b
PUFA [*]	61.36 ± 0.41 ^a	61.25 ± 0.09 ^a	61.18 ± 0.25 ^a
Polyene Index [†]	4.11 ± 0.03 ^a	4.00 ± 0.03 ^b	4.06 ± 0.04 ^{ab}
Lovibond Red	0.3 ± 0.1 ^a	0.6 ± 0.0 ^b	0.8 ± 0.1 ^b
Lovibond Yellow	0.8 ± 0.1 ^a	2.3 ± 0.2 ^b	3.1 ± 0.2 ^c
Peroxide Value (meq/kg)	0.85 ± 0.11 ^a	7.33 ± 1.46 ^b	5.40 ± 1.59 ^c
Free Fatty Acids(%)	0.07 ± 0.01 ^a	0.10 ± 0.01 ^b	0.18 ± 0.01 ^c
OSI (hrs)	6.12 ± 0.12 ^a	4.20 ± 0.09 ^b	4.50 ± 0.10 ^c
Total Tocopherols (mg/100g)	94.20 ± 0.55 ^a	81.93 ± 0.09 ^b	85.32 ± 0.63 ^c
Cholesterol (mg/100g)	<1.00 ^a	2.29 ± 0.53 ^b	3.09 ± 0.25 ^c
Campesterol (mg/100g)	65.25 ± 6.18 ^a	31.41 ± 2.68 ^b	34.16 ± 0.79 ^b
Stigmasterol (mg/100g)	47.11 ± 5.00 ^a	28.85 ± 2.50 ^b	31.61 ± 0.90 ^b
B-sitosterol (mg/100g)	147.06 ± 15.86 ^a	96.47 ± 9.08 ^b	99.50 ± 2.32 ^b

^{*}Percent of total fatty acids.

[†]Ratio of PUFA to saturated fatty acids.

Values followed by the same letter were not found to differ significantly for each row using Duncan's multiple range test (p<0.05) (n=3).

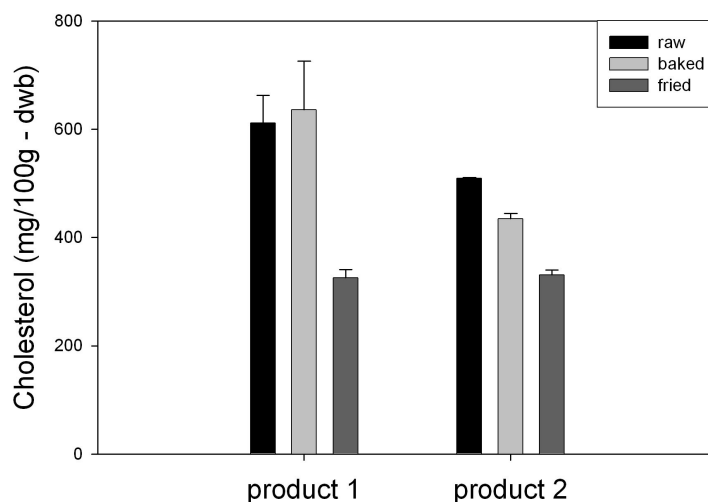


Fig. (2). Cholesterol content in shrimp portions. Values in mg cholesterol per 100 g sample on a dry weight basis (dwb).

Both cholesterol and phytosterols are oxidized by heat [8]. Cooking oils and Product 1 were analyzed by GC-MS for oxidation products. Only one breaded shrimp product was analyzed, as this study sought to explore the feasibility of this type of analysis in a complete food system and this product was seen to have a decrease in sterol content compared to Product 2. Several studies in the literature include spectral data and were compared to the spectra obtained here to tentatively identify the oxides present [8, 14, 28-30]. Oxidation products of cholesterol and phytosterols were detected in the oil samples used for frying the breaded shrimp products and tentatively identified based on their mass spectra.

The compounds found were epoxycholesterol, an ethylcholest-4-ene- β -diol, 24-hydroxysitosterol, 4- β -hydroxysitosterol, cholestane-3 β , 5,6- β -triol, 4- β -hydroxystigmasterol, 7- α -hydroxysitosterol, 4- β -hydrocholesterol and stigmasterol triol. The amounts of the compounds were not quantified due to lack of authentic standards. Other studies have assumed a general relative response factor of 1 for quantification in the absence of standards [14]. Taking this into account, the amounts of these oxides would be between 1 and 10 ppm in the oils. Based on the relative response factor of 1, the level of detection (0.1 mg/100g) was used in this analysis. These limits are in agreement with similar studies [30].

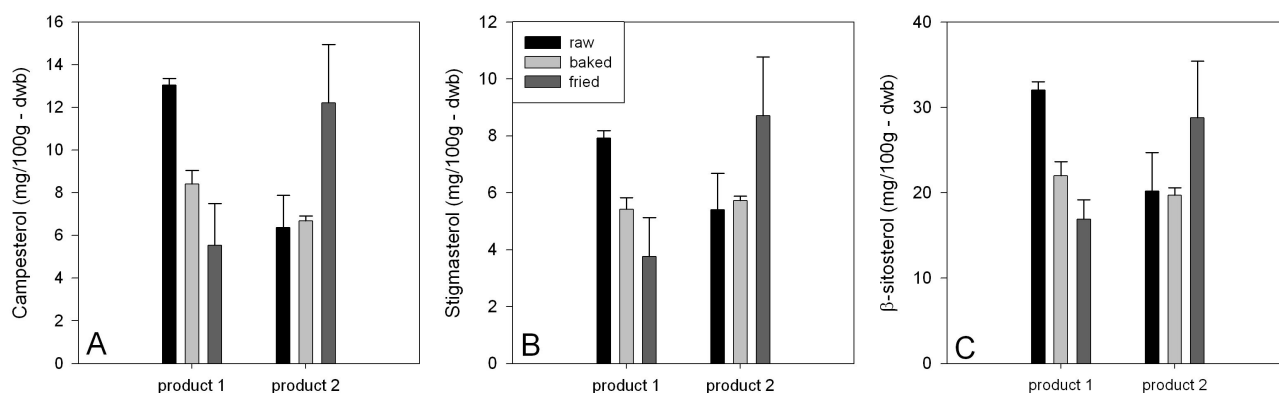


Fig. (3). Phytosterol content of breaching portions. **A.** Campesterol, **B.** Stigmasterol, **C.** Beta-sitosterol.

The presence of COPS in the cooking oils demonstrated the transfer of these products from the cholesterol containing food to the frying matrix. As the oil chosen was cholesterol free (Table 2), the fried breaded shrimp were attributed to be the source of the COPS due to their high levels of cholesterol. COPS may have migrated directly from the cooked breaded shrimp into the hot oil or may have formed in the frying oil *via* oxidation of cholesterol that was transferred to the hot oil from the breaded shrimp during frying.

Mass spectral comparisons showed COPS but no POPS in the shrimp or breaching portions. There are so many phytosterol oxidation products possible; any produced may have been below the detection limits of the instrumentation used. The cholesterol oxides identified were 7α -hydroxycholesterol, 5α - 6α -epoxycholesterol, 24-hydroxycholesterol and 20-hydroxycholesterol. These compounds were found in the baked and the fried shrimp portions. Another compound was found in the baked shrimp portion that appeared to have the same ring structure as cholesterol but without the side chain based on the molecular weight. This indicates further degradation of the cholesterol is occurring under the cooking conditions. Much of the work with cytotoxicity of COPS and POPS has been done at levels of mmol/L which is well above those seen in this study [31]. From our results, it was seen that these compounds are produced even with the short cooking times for consumer packages with no oil reuse that would be seen in a commercial setting. The importance of this will be determined as more work is done to determine the levels of toxicity and if the compounds are found to be cumulative. Exposure to condensed volatile emissions from cooking oils over time has been found to be toxic [32]. It may be that more attention needs to be paid to other lipid oxidation products as well.

CONCLUSION

The frying of commercially prepared frozen breaded shrimp products in soybean oil for 4 min resulted in oxidative deterioration both in the cooked products and the frying oils. Also, the heating of cholesterol containing foods under conditions that a normal consumer would use promoted oxidation of the cholesterol in the food, as evidenced by the breaded shrimp models. As the safety of oxidized sterols has been called into question, it may be important to note that ordinary methods of food preparation result in detectable amounts of sterol oxides and other lipid oxidation products

in the final product [33, 34]. Reuse of cooking oil may result in accumulation of COPS and POPS and allow them to be transferred to food.

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