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Interaction between soybean oil and the lipid fraction of fried pitu prawn

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ABSTRACT

The interaction between the soybean oil used to fry pitu prawn and the lipid fraction was assessed from the changes on its composition of fatty acids, cholesterol content and the formation of cholesterol oxides. The proximate composition, caloric value and nutritional quality were determined. Frying in soybean oil increased the lipid, calories and n-6 polyunsaturated fatty acids (n-6 PUFA) contents and reduced the n-3 PUFA, consequently increasing n-6/n-3 ratio. Cholesterol and its oxidation products remained unchanged after frying. The ratio of hypocholesterolemic:hypercholesterolemic fatty acids (HH) observed in raw and fried samples was high and the values of the atherogenicity (AI) and thrombogenic indexes (TI) were low and decreased after frying. The fried pitu prawn can be considered a good source of protein, monounsaturated fatty acids (MUFA), n-6 PUFA, n-3 PUFA, especially eicosapentaenoic acid (EPA), and low in cholesterol and cholesterol oxides.

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1. Introduction

Crustaceans like shrimps are a good source of long chain n-3 PUFA, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Tsape, Sinanoglou, & Miniadis-Meimaroglou, 2010). For this reason, shrimps consumption has been encouraged since there is an association between n-3 PUFA, the promotion of human health and reduction of risks of cardiovascular diseases due to its action in various physiological and metabolic processes, such as the maintenance of biological membranes (Simopoulos, 2008).

The n-3 PUFA are also precursors of eicosanoids with anti-inflammatory, antithrombotic and vasodilator properties, which are related to the prevention of some diseases, such as dyslipidemia and hypertension (Connor, 2000). On the other hand, the n-6 PUFA, such as linoleic acid and arachidonic acid, are precursors of eicosanoids with pro-inflammatory and pro-aggregatory properties and thus, when in excess, they induce an inflammatory state, which is the basis to the development of chronic diseases, e.g. coronary heart disease (CHD), diabetes, arthritis, cancer and osteoporosis (Simopoulos, 2008). Moreover, Western diets are defined by the intake of high contents of n-6 PUFA and low contents of n-3 PUFA. The currently estimated n-6 PUFA to n-3 PUFA ratio in the diet ranges from 15:1 to 20:1, bearing in mind that recommendations would be from 5:1 to 10:1 by the Food and Agriculture Organization (FAO, 2010).

Frying is a food processing method used to prepare both homemade and industrial products, which is widespread due to the sensory and organoleptic characteristics that make fried food more attractive to consumers. However, during this process, lipids can undergo oxidation, resulting in reduced concentrations of n-3 PUFA and increased cholesterol oxides content (Echarte, Zulet, & Astiasarán, 2001). Crustaceans present high PUFA and cholesterol content in their lipid fraction (Bragagnolo & Rodriguez-Amaya, 2001; Lira et al., 2007), favouring the oxidation of cholesterol, which results in the production of cholesterol oxides, biologically active molecules that are related to cytotoxic, teratogenic and mutagenic effects (Saldanha, Benassi, & Bragagnolo, 2008).

Despite the considerable interest in n-3 PUFA, n-6 PUFA, cholesterol and cholesterol oxides, little research has been conducted to detect possible changes that food may suffer when fried. Seeking to fill this gap in the scientific literature, the aim of this work was to assess the interaction between soybean oil and the lipid fraction of fried pitu prawn (*Macrobrachium acanthurus*, Wiegman, 1836) by analysing the changes in fatty acids, cholesterol and cholesterol oxides content. The proximate composition, caloric value and nutritional quality were also assessed since pitu prawn is a commercially relevant hand extracted crustacean which is considerably consumed in the northeast region of Brazil.

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2. Materials and methods

2.1. Samples

Twelve pitu prawn samplings were made in Lagoon-Estuary Complex Mundau/Manguaba, located in the state of Alagoas, Brazil, between January and March 2009. The water temperature ranged from 25 °C to 30 °C and the average salinity was 21 g/100 g. Each sample consisted of 400 g of pitu prawn, which were packed in plastic bags, kept on ice and immediately transported to the laboratory. After exoskeleton, cephalothorax and intestine removal, the prawn meat was separated into two groups of 150 g. Group 1 composed the raw samples and group 2, the fried samples. The prawns were fried using 50 mL of soybean oil for 5 min at 160 °C. After frying, the prawns were placed on an absorbent paper to remove oil excess. Both samples, *i.e.* raw and fried, were homogenised in a blender and convenient aliquots were weighted in duplicate to perform the analyses.

2.2. Methods

2.2.1. Proximate composition

The determinations of moisture, ash and protein were carried out according to the AOAC methods (1990). Total lipids were determined gravimetrically according to Folch, Lees, and Stanley (1957). The carbohydrate quantification was performed by difference considering the contents of moisture, ash, proteins and lipids. The results were expressed as g/100 g of prawn in wet basis. Total caloric value was calculated using the caloric coefficients for proteins, lipids and carbohydrates (Livesey, 1990).

2.2.2. Fatty acid composition

The lipids (25 ± 1 mg) obtained according to Folch et al. (1957) were converted into methyl esters (Hartman & Lago, 1973) and injected on to a gas chromatograph (GC). The GC (Shimadzu, Kyoto, Japan) was equipped with a split injector (1/50) at 250 °C, fused-silica capillary column (100 m of length, 0.25 mm of i.d., 0.20 μm of thickness stationary phase, CP-SIL 88, Chromopack, Middleburg, The Netherlands), flame ionization detector at 260 °C and workstation (GCSolution, Shimadzu, Kyoto, Japan). The temperature was programmed starting at 120 °C during 8 min, increasing to 160 °C at 20 °C/min, remaining at 160 °C for 4 min, increasing from 160 °C to 195 °C at 3 °C/min remaining at 195 °C for 10 min, increasing from 195 °C to 220 °C at 35 °C/min, remaining at 220 °C and after 3 min, it went up to 240 °C at 20 °C/min and remained for 5 min, totalling 46 min (Sancho, Lima, Costa, Mariutti, & Bragagnolo, 2011). Carrier gas was hydrogen with a linear velocity of 34 cm/s and make-up gas was nitrogen with a flow of 30 mL/min. The injection volume was 2 μL and the hot-needle technique for 5 s was applied. The identification of methyl esters was performed by comparing the retention times of standards peaks with the retention times of sample peaks. A mixture of standards containing methyl esters of fatty acids from 4:0 to 24:0 (FAME Mix C4-C24, Supelco, Bellefonte, Pennsylvania, USA and FAME 22:4n-6, Sigma–Aldrich, St. Louis, MO, USA) were used to identify the fatty acids. The fatty acids quantification was done by normalisation and the results expressed in mg/100 g of edible portion, using the conversion factor for crustaceans described by Exler (1975) and Weihrauch, Posati, Anderson, and Exler (1977).

2.2.3. Cholesterol and cholesterol oxides

The determination of cholesterol and cholesterol oxides was carried out according to Saldanha, Sawaya, Eberlin, and Bragagnolo (2006) with some modifications. The modifications were: sample weight (1.0 ± 0.1 g), number of extractions of unsaponifiable matter (4 extractions, 10 mL hexane) and an additional wash step of the hexane extract with 5 mL 0.5 mol equiv/L aqueous KOH solution and four washes with 5 mL distilled water. The extract was dried in a rotator evaporator, redissolved in mobile phase filtered through a membrane (0.45 μm, Millipore) and injected into the high performance liquid chromatography (HPLC).

A liquid chromatography (Shimadzu, Kyoto, Japan) with UV–visible (SPD-10 AVvp) and refractive index (RID-10 A) detectors connected in series and manual injector with a 20 μL loop was used. The analytical column was Nova-Pack CN HP (300 mm × 3.9 mm × 4 μm, Waters, Milford, USA) and the temperature was set at 32 °C. A mobile phase of hexane and 2-propanol (97:03, mL:mL) with an isocratic flow rate of 1 mL/min was used to separate cholesterol from the following oxides: 19-hydroxycholesterol, 20-hydroxycholesterol, 22*S*-hydroxycholesterol, 25-hydroxycholesterol, 5,6α-epoxycholesterol, 5,6β-epoxycholesterol, 7-ketocholesterol, 22*R*-hydroxycholesterol, 24*S*-hydroxycholesterol, 26-hydroxycholesterol, 7α-hydroxycholesterol and 7β-hydroxycholesterol. To separate cholestanotriol, hexane and 2-propanol (90:10, mL:mL) was used with a flow rate of 1 mL/min. The analysis time was 50 min to elute all the compounds.

The identification of cholesterol and cholesterol oxides was performed by comparing the retention times of the samples with standards and by co-chromatography. The cholesterol (5-cholesten-3-ol) and cholesterol oxides standards: 19-hydroxycholesterol, 20-hydroxycholesterol, 22*S*-hydroxycholesterol, 25-hydroxycholesterol, 5,6α-epoxycholesterol, 5,6β-epoxycholesterol, cholestanotriol and 7-ketocholesterol were purchased from Sigma (Milford, Mass., USA) and 22*R*-hydroxycholesterol, 24*S*-hydroxycholesterol, 26-hydroxycholesterol, 7α-hydroxycholesterol and 7β-hydroxycholesterol were acquired from Steraloids (Newport, R.I., USA). The purity of the standards ranged from 95 to 98%. Hexane and 2-propanol for chromatograph were HPLC grade, degassed and filtered, while the other reagents were analytical grade. The cholesterol oxides were confirmed by HPLC–APCI–MS–MS by comparison of the mass spectra in the retention times of the authentic standards with the respective cholesterol oxide peak in the sample (Mariutti, Nogueira, & Bragagnolo, 2008).

2.2.4. Indexes of nutritional quality of lipids

Nutritional quality of lipid fraction was assessed from fatty acids composition. The PUFA/SFA and n-6/n-3 PUFA ratios, atherogenicity (AI) and thrombogenic indexes (TI) (Ulbricht & Southgate, 1991) and hypocholesterolemic:hypercholesterolemic (HH) fatty acids ratio (Santos-Silva, Bessa, & Santos-Silva, 2002) were calculated.

2.2.5. Statistical analysis

The experiment was conducted in a completely randomised design, with two treatments (raw and fried prawn). The data were subjected to ANOVA by *F*-test and the averages were compared by Student's *t*-test. In this case, the study involved only two treatments, so the *F*-test was equivalent to Student's *t*-test ($t^2 = F$) (Steel & Torrie, 1981). The analyses were performed using the SAEG – System for Statistical Analysis Software (2007).

3. Results and discussions

3.1. Effects of soybean oil in the proximate composition

The results of chemical composition analyses of raw and fried pitu prawn, calculated on wet basis, are presented in Table 1. Moisture content was 74 ± 2 g/100 g in raw pitu prawn and decreased significantly ($p < 0.01$) after frying (68 ± 1 g/100 g). The increase in the total lipid concentration in fried fish was also observed by Ersoy and Ozeren (2009), Weber, Bochi, Ribeiro,

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