Determination of Sterols in Sea Urchin Gonads by High-Performance Liquid Chromatography With Ultraviolet Detection

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Abstract

A reversed-phase high-performance liquid chromatography (HPLC) method for the determination of sterols in sea urchin *(Paracentrotus lividus)* gonads is described. After saponification with KOH, samples are extracted with hexane, evaporated to dryness, and dissolved in HPLC eluant. Sterols were separated using a C_{18} column as the stationary phase, methanol–acetonitrile (30:70, v/v) as the mobile phase, and ultraviolet detection at 205 nm. Three sterols were identified and quantitated: desmosterol, fucosterol, and cholesterol. Relative standard deviations (%) were 2.32 for desmosterol, 2.31 for fucosterol, and 2.13 for cholesterol. Limits of detection were 2.31, 0.56, and 2.33 μg/mL for desmosterol, fucosterol, and cholesterol, respectively.

Introduction

The sea urchin is a shellfish belonging to the *Echinoidea* family that settles in groups between rocks, stones, and algae at a depth of 20 m. This shellfish is bowl-shaped, dark in color, and 7–8 cm in diameter with a shell covered with spines (1). More than 750 species of sea urchins have been identified, of which *Paracentrotus lividus* is the most appreciated sea urchin. Sea urchins of this species grow in Atlantic and Mediterranean coasts, and they are very plentiful in Galicia (Atlantic coast of Spain) with an annual harvest exceeding 1000 tons (2).

The sea urchin has been favored by consumers because of its distinctive aroma and good taste. The edible portions are the 5 gonads, which are half-moon-shaped and yellow-orange in color and constitute only 10% of the total weight. The season for sea urchin is from the beginning of January to the middle of April when the development of gonads is at its maximum (3,4). Gonads are eaten fresh in sauces, soups, creams, and omelettes; however, due to the seasonality and high perishability of gonads, they are generally processed into a canned product known as sea-urchinroes.

Sterols are the main constituents of the unsaponifiable matter and represent an important group of minor components of oils and greases (5). Cholesterol is the most abundant sterol found in animal products. In comparison with fish, the lipid content of shellfish is low, whereas the cholesterol content is relatively high (6). Much attention has been focused on the levels of cholesterol in different foods, because high levels of cholesterol are related to cardiovascular disease (7). On the other hand, cholesterol is the precursor to steroid hormones, bile acids, and Vitamin D and is also necessary for the formation of biological membranes (8).

Presently, normal and reversed-phase high-performance liquid chromatography (RP-HPLC) methods are used to evaluate sterols in foods using an ultraviolet (UV) detector (9), refractive index detector (10), or light-scattering detector (11). On normal silica phase, distinct product classes are separated, but individual components within a group (for example, cholesterol and phytosterols) remain unresolved (12). In this work, an RP-HPLC method for determination of sterols in sea urchin gonads using UV detection at 205 nm is described.

Experimental

Samples

Sea urchins were caught off the Atlantic coastal region around Touriñán in Galicia (northwest Spain) in March, 1998. After the catch, the gonads were separated using a knife, kept on ice, and immediately transported to a canning factory (Conservas y Ahumados Lou, Ribeira, La Coruña, Spain). Gonads (90 g) were placed in an RR-90 can, and salt and tap water were added. The cans were vacuum-sealed and sterilized at 112°C for 50 min.

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Fresh gonads were also transported to the laboratory and immediately analyzed.

Reagents

Cholesterol, fucosterol, and desmosterol standards were obtained from Sigma Chemical (Madrid, Spain). HPLC-grade acetonitrile and methanol were from Scharlau (Barcelona, Spain). Reagent-grade potassium hydroxide was from Vorquímica (Vigo, Spain). Ethanol was from Normasolv (Barcelona, Spain).

HPLC apparatus

The HPLC equipment consisted of a Spectra Physics (San Jose, CA) HPLC apparatus comprised of an SP8800 ternary pump, a 20-µL Rheodyne (Cotati, CA) injection loop, an SP8792 column heater, a UV–visible forward optical scanning detector linked via Labnet to a Pentium computer running PC-1000 version 3.0 software (all from Thermo Separation Products, Fremont, CA) on IBM OS/2 Warp version 3.0 hardware. A column (250 × 4.6 mm) packed with 5-µm Spherisorb C_{18} (Sugelabor, Madrid, Spain) was used for the separation.

Sample preparation

A sample (10 g) was weighed in an Erlenmeyer flask and saponified by refluxing and stirring for 30 min with 100 mL of 1M ethanolic KOH. After cooling to room temperature, the mixture was transferred to a separatory funnel with 50 mL of hexane, and the funnel was stoppered and shaken for 3 min. After allowing the layers to separate, the lower layer was run into the Erlenmeyer flask, and the top layer was collected in a 100-mL flask. The lower layer was placed in the funnel and extracted twice more with 25 mL of hexane each time; all of the solvent extracts were pooled and made up to 100 mL. A portion (10 mL) of this extract was evaporated to dryness under a nitrogen stream, and this residue was redissolved in 2 mL of HPLC eluant, filtered through a 0.45-µm membrane filter (Millipore, Bedford, MA), and injected into the chromatograph.

Chromatographic procedure

The analysis was carried out at a constant temperature of 35°C with isocratic elution using methanol–acetonitrile (30:70, v/v) at a flow rate of 1 mL/min. UV absorbance spectra were obtained in the range of 200–300 nm. The detection wavelength selected for sterol quantitation was 205 nm, which afforded the greatest response.

Results and Discussion

In developing the method, several changes in the proportions of the mobile phase components were assayed (50:50, 40:60, and 30:70 methanol–acetonitrile), and several flow rates were tried (1, 1.2, and 1.5 mL/min). All mobile phases at different flow rates gave a good resolution of desmosterol and cholesterol, but only the 30:70 mixture at a flow rate of 1 mL/min allowed the adequate resolution of fucosterol. A good resolution of the 3 sterols below these conditions is obtained at room temperature, but when analysis is performed at 35°C, the analysis time is reduced (from 40 to 30 min).

Figures 1 and 2 show the chromatograms of the sterol standard mixture and a typical sea urchin sample, respectively. The identification of the sterols was made by comparing their retention times and UV absorbance spectra with those of standards of known concentration. Three sterols were identified and quantitated: desmosterol, fucosterol, and cholesterol. Cholesterol and desmosterol were selected as standards because they have been identified as the major sterols in *Paracentrotus lividus* of the Adriatic sea (13); fucosterol was selected because it is the most abundant sterol in brown algae (14).

To determine the precision of the method, 6 aliquots of the same homogenized sea urchin sample were subjected to the complete procedure and injected in duplicate. The relative standard deviations (RSDs, %) were 2.32, 2.31, and 2.13 for desmosterol, fucosterol, and cholesterol, respectively.

Recovery percentages were evaluated by spiking samples of sea urchin with a mixed standard and then subjecting them to the

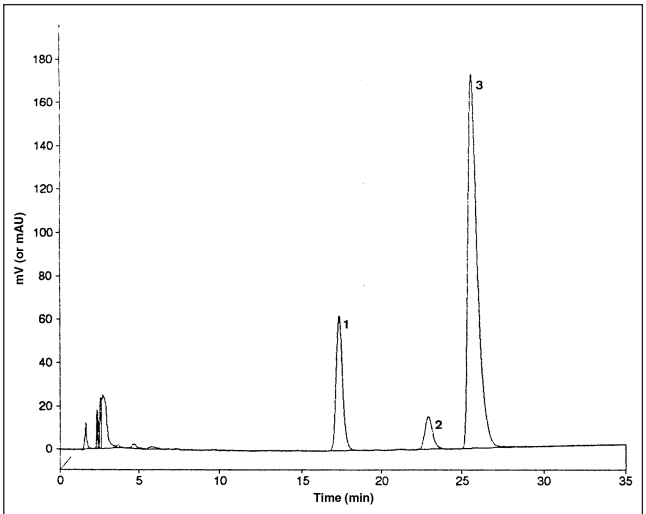

Figure 1. Chromatogram of a sterol standard. Peaks: 1, desmosterol; 2, fucosterol; 3, cholesterol.

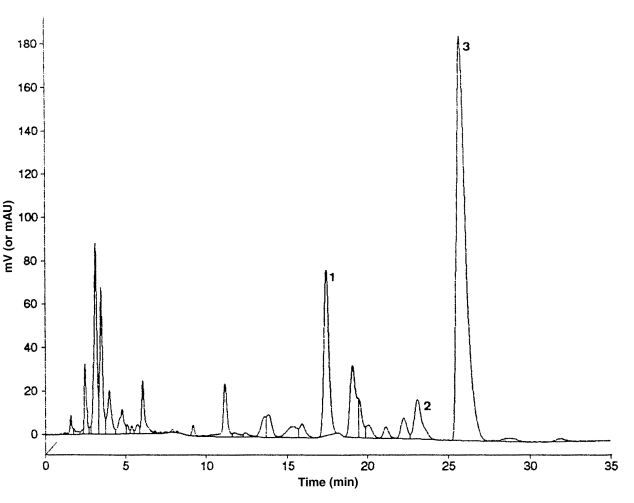

Figure 2. Chromatogram of a sea urchin gonad sample. Peaks: 1, desmosterol; 2, fucosterol; 3, cholesterol.

Table I. Sterol Contents (mg/100 g dry weight) in Raw and Canned Sea Urchin Gonads

Sterol	X ± SD* Raw	Canned
Desmosterol	70.9 ± 12.33	66.3 ± 2.53
Fucosterol	89.4 ± 9.94	85.6 ± 6.40
Cholesterol	833 ± 39.1	873 ± 67.7

* Mean ± standard deviation (4 samples).

rest of the procedure. Recovery percentages were higher than 91.3%.

The detection limits, calculated in accordance with the American Chemical Society guidelines (15), were 2.31, 0.56, and 2.33 μg/mL for desmosterol, fucosterol, and cholesterol, respectively.

The method was calibrated using a series of sterol standards (4 concentration levels in the range of analytical interest). Correlation coefficients for these data exceed 0.996.

Cholesterol was also determined enzymatically using a commercial test kit for cholesterol analysis (Boehringer-Mannheim number 139 050) (16). Student's *t* test (using Stat-Graphics package version 2.6) showed no significant difference ($p \leq 0.05$) between the mean of the 6 subsamples determined by the HPLC method and the enzymatic method, which gave an RSD of 3.01%.

The predominant sterol in sea urchin gonads was cholesterol (83.9%), followed by fucosterol (9.0%) and desmosterol (7.13%). The presence of fucosterol, a vegetal sterol, is related to the diet of the sea urchin (13,17), which usually feeds on brown algae (Laminaria spp.) (18). Desmosterol is the metabolic intermediate in the process of transforming the vegetal sterols into cholesterol (19). The results confirm that in *Paracentrotus lividus*, as well as in other echinoid species, 5 sterols are prevalent (20). In Table I, the concentrations of sterols in raw and canned sea urchin gonads are given. The *t* test comparing the mean sterol contents of raw gonads and gonads sterilized at 112°C for 50 min indicated that differences were not significant ($p \leq 0.05$).

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