STEROL COMPOSITION OF SHELLFISH COMMONLY CONSUMED IN THE UNITED STATES

K.M. PHILLIPS[1], D.M. RUGGIO[1], K.R. AMANNA[1], K.Y. PATTERSON[2], and J. EXLER[2]

[1]Virginia Tech, Blacksburg, VA , [2]USDA , ARS, Beltsville, MD

USDA ARS Beltsville Human Nutrition Research Center

Serving The Nation Since 1906. Improving Health Thru Research.

Abstract

Objective: The goal was to obtain quantitative data on the sterol content and composition of shellfish and mollusks commonly consumed in the U.S. to update the USDA National Nutrient Database for Standard Reference, using a nationwide sampling plan and validated analytical methodology.

Materials and Methods: In 2007-8, the USDA sampled raw shrimp and sea scallops; steamed oysters, blue crab, and lobster; and clams (canned) from 12 statistically selected supermarkets across the United States. Raw mussels and clams were sampled locally (Blacksburg, VA). For each species, four composites comprising 3 locations were prepared; shrimp and scallops from 6 single locations were also analyzed. Fourteen sterols, major and minor, were determined in total lipid extracts after saponification and derivatization, using GC-FID for quantitation and MS for confirmation of peak identities.

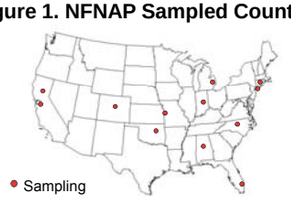
Results: Crab, lobster, and shrimp contained significant levels (96.2-127 mg/100g fresh wt) of cholesterol; canned clams and scallops had the lowest concentrations (23.4-30.1). Variability in cholesterol among single-location samples of shrimp was low. The major sterols in the mollusks were brassicasterol (12.6-45.6 mg/100g) and 24-methylenecholesterol (16.7-41.9), with the highest concentrations in oysters. Total non-cholesterol sterols were 46.5-75.6 in five scallops samples, but 107 mg/100g in one, with cholesterol also higher in that sample.

Significance: Crustaceans contain significant amounts of cholesterol and a high ratio of cholesterol/non-cholesterol sterols, while the opposite was found for mollusks. Between-sample variability in sterol content for some species suggests average concentrations may not represent those in specific local samples and demonstrates the importance of a representative sampling plan to obtaining reliable food composition data.

Introduction

- Cholesterol has been the only sterol reported for shellfish in the USDA National Nutrient Database for Standard Reference, through the current release, SR23; however, there are over 12 other sterols present in shellfish in amounts greater than trace levels.
- Numerous literature reports on shellfish sterol composition are based on limited sampling, with studies done mostly for comparative physiology or local food composition analyses.
- There is no report on the sterols (content and composition) of commonly consumed shellfish from the U.S. retail market.
- Accurate values and estimates of variability are essential to generate reliable data to estimate cholesterol and other sterols in the food supply.
- Given the research interest in health effects of dietary cholesterol and non-cholesterol sterols, such data are required to support epidemiological studies and feeding trials.

Figure 1. NFNAP Sampled Counties



Materials and methods

Sampling

A nationally representative sampling of three crustaceans (crab, lobster, and shrimp) and three mollusks (clams, oysters, and scallops) was obtained.

- Sample units were procured from 12 supermarkets across the country in 2007-2008 (Fig. 1), as part of the National Food and Nutrient Analysis Program (NFNAP)[1,2]. For convenience of handling, the oyster, crab and lobster sample units were obtained steamed.
- Three or four composites of each species were prepared and analyzed, with each composite comprising three locations.

Sample Preparation

- All samples were shipped on dry ice after purchase to the Food Analysis Laboratory Control Center (FALCC) at Virginia Tech, where they were stored frozen (≤ -15ºC) prior to compositing, except canned clams, which were shipped and stored at ambient temperature.
- Samples were cleaned of inedible portions, composited, and homogenized with liquid nitrogen using standard protocols previously established to insure homogeneity and to preserve nutrient integrity[3].
- Subsamples were dispensed into 1-oz. jars, sealed under nitrogen, and stored frozen at ≤ -60ºC until analyzed.

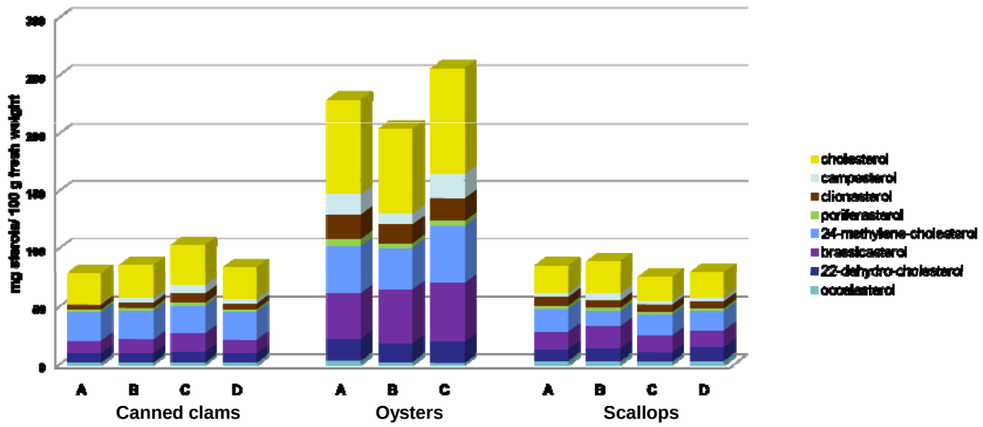
Nutrient Analysis

- Total lipid was extracted with chloroform/methanol[4] and sterols were analyzed in total lipid extracts using previously reported methodology, involving alkaline saponification and gas chromatographic analysis of the trimethylsilylether derivatives, with epicholesterol as an internal standard[5].
- Mass spectrometry of selected samples was performed to confirm identify of sterols in addition to the relative retention times.

Quality Control

An in-house prepared control material, Salmon Control Composite, was analyzed with samples[6]. The target value and uncertainty range for cholesterol in this material had been established after co-analysis with the National Institute of Standards and Technology SRM 1546 Meat Homogenate and by multiple analytical values from three independent labs.

Figure 2. Sterols in mollusk sample composites A, B, C, and D[a]



[a] Each composite was made up of three locations

Table 1. Sterol content of crustaceans and mollusks, mean (SD) in mg/100 g

Seafood	n[a]	occelasterol	22-dehydro-cholesterol	brassicasterol[b]	desmosterol/7-dehydrocholesterol[c]	24-methylene-cholesterol	poriferasterol	clionasterol	campesterol	cholesterol
Lobster, steamed	3	<0.1	2.49 (0.68)	---	1.39 (0.42)	0.08 (0.14)	<0.1	0.14 (0.12)	0.26 (0.08)	146 (14)
Shrimp, raw	4	<0.1	1.27 (0.17)	---	0.44 (0.04)	0.21 (0.04)	0.3 (0.09)	0.66 (0.24)	1.16 (0.4)	127 (7)
Crab, steamed	3	<0.1	1.52 (0.71)	---	1.41 (1.42)	0.70 (0.06)	<0.1	0.30 (0.04)	1.43 (1.0)	96 (30)
Clams, canned	4	1.78 (0.04)	8.49 (0.86)	12.9 (2.6)	---	24.3 (0.7)	2.52 (0.23)	5.7 (2.07)	3.56 (3.49)	30 (4)
Oyster, steamed	3	2.42 (0.97)	17.8 (1.4)	45.6 (5.7)	---	41.9 (6.7)	4.80 (1.11)	19.2 (2.4)	16.1 (6.0)	82 (9)
Scallops, raw	4	2.98 (0.35)	9.64 (1.42)	16.3 (2.6)	---	16.7 (3.5)	2.83 (0.35)	6.72 (0.7)	4.33 (1.84)	24 (3)

[a] Each sample composite is comprised of sample units from three locations; [b] May contain trace amounts of desmosterol/7-dehydrocholesterol; [c] May contain trace amounts of brassicasterol

Discussion

- Cholesterol levels in crustaceans are relatively higher than in mollusks. The lowest level of cholesterol in crustaceans (96 mg/100g in crab) is higher than the highest level in mollusks (82 mg/100g in oysters).
- Levels of total non-cholesterol sterols in mollusks are 2-3 times that of cholesterol, ranging from 70.9 to 181 mg/100g fresh wt.
- Mollusks have a higher content than crustaceans of sterols, primarily brassicasterol and campesterol.
- Between-sample variability in sterol content is greatest in oysters (Fig. 2).
- In contrast, lobster and shrimp showed little variability between samples. Most of the sterol content was cholesterol with a %RSD of 9 and 5%, respectively. Crab was higher at 31%.
- Mass spectrometry was critical to distinguish sterols in some cases, particularly brassicasterol (in mollusks) vs. desmosterol in crustaceans, due to overlapping retention times on GC.

Conclusions

- For the mollusks, cholesterol was the most abundant sterol but several other sterols were present in significant amounts, particularly in oysters.
- For the crustaceans, the predominate sterol was cholesterol with only minor amounts of other sterols.
- Between-sample variability demonstrates the importance of a representative sampling plan to obtaining reliable food composition data applicable to national surveys.
- Data for sterols will be released in SR24, summer 2011- available at the NDL Web site http://www.ars.usda.gov/nutrientdata); results for other nutrients (vitamins, minerals, proximates, fatty acids, amino acids) analyzed in the same composites were included in SR23, released in September 2010.

References

1 Pehrsson, P.R.; Haytowitz, D.B.; Holden, J.M.; Perry, C.R.; Beckler, D.G. USDA's National Food And Nutrient Analysis Program: Food sampling. *J. Food Comp. Anal.* 2000,13, 379-389.

2 Perry, C.R.; Pehrsson, P.R.; Holden, J.M. A revised sampling plan for obtaining food products for nutrient analysis for the USDA National Nutrient Database. *Proc. Am. Stat. Assoc.* 2003, [serial online]. Available from American Statistical Association (http://www.amstat.org/Sections/Srms/Proceedings/y2003f.html). Accessed March 18, 2010.

3 Trainer, D.; Pehrsson, P.R.; Haytowitz, D.B.; Holden, J.M.; Phillips, K.M.; Rasor, A.S.; Conley, N.A. Development of sample handling procedures for foods under USDA's National Food and Nutrient Analysis Program. *J. Food Comp. Anal.* 2010, 23, 843-851.

4 Phillips, K.M.; Ruggio, D.M.; Amanna, K.R. Extended validation of a simplified extraction and gravimetric determination of total fat to selected foods. *J. Food Lipids*, 2008, 15:309-325.

5 Phillips, K.M.; Ruggio, D.M.; Ashraf-Khorassani, M. Phytosterol composition of nuts and seeds commonly consumed in the United States. *J. Agric. Food Chem.*, 2005, 53:9436-9445.

6 Phillips. K.M.; Patterson, K.Y.; Rasor, A.R.; Exler, J.; Haytowitz, D.M.; Holden, J.M.; Pehrsson, P. R. The role of quality control and reference materials in the National Food and Nutrient Analysis Program. *Anal. Bioanal. Chem.*, 2006, 384:1341-1355.

Acknowledgement. This work was supported by the USDA Agricultural Research Service. Partial support was received from the National Institutes of Health, agreement #Y1CN5010, and from the FDA.