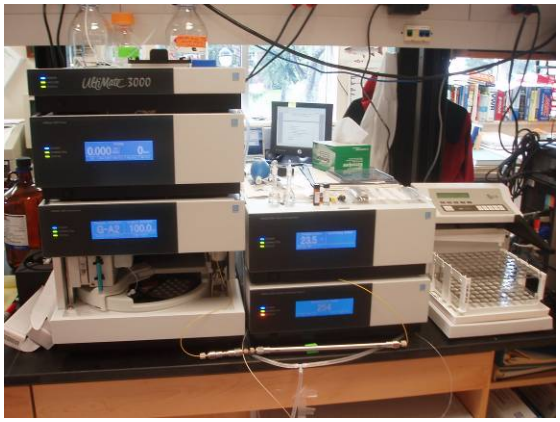
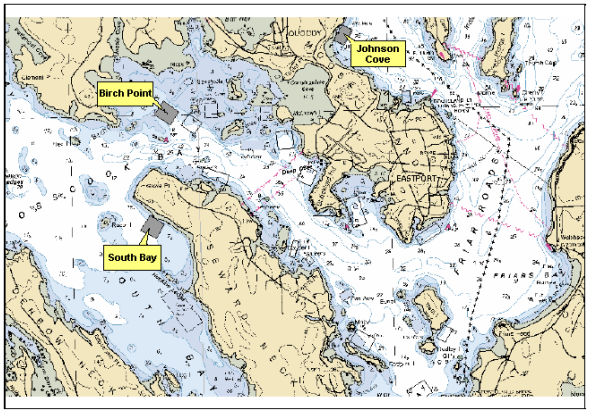
Analysis of Carotenoids in Sea Urchins

Brian Perkins, Ph.D.
Laboratory Director & Assistant Research Professor
Food Chemical Safety Laboratory, Department of Food Science and Human Nutrition
200 Hitchner Hall
University of Maine Orono, ME 04469

August 2008

Photos above by Chris Bartlett





Project
Analysis of Carotenoids in Sea Urchins for the Maine Department of Marine Resources (DMR)

Objective
The goal of this project was to detect and quantify canthaxanthin extracted from the roe of sea urchins. The urchin samples were collected by DMR divers from pre-determined transects in the proximity of pens used for salmon aquaculture. Approximately 100 (live) urchins (10 animals from 11 collection sites) were delivered to the Food Chemical Safety Laboratory at the University of Maine in the Fall of 2007. The samples were stored at -80 degrees C until they were analyzed. Because our laboratory was also doing work with astaxanthin, it was decided to analyze the urchins for this carotenoid, as well.

Methodology (modified from Japan Ministry of Health, Labour, and Welfare, Department of Food Safety)

A. Sample Preparation
    1. Homogenize 5.0 g of sample (roe) with 30 mL acetonitrile, 20 mL n-hexane and 10 g sodium sulfate.
    2. Centrifuge homogenate for 5 min at 3000 rpm.
    3. Transfer acetonitrile and hexane fractions to a 125 mL separatory funnel collect the acetonitrile (lower) fraction.
    4. Add another 20 mL acetonitrile and the residual hexane fraction to the centrifuge pellet and re-homogenize.
    5. Centrifuge mixture again, at 3000 rpm and discard hexane fraction.
    6. Mix the two acetonitrile fractions together and add 10 mL n-propanol.
    7. Concentrate the resulting solution to 5 mL under nitrogen at 40 degrees C using a TurboVap (Zymark, Inc., Hopkinton, MA).
    8. Bring final evaporated sample to 10 mL (volumetric flask) with HPLC grade methanol.

B. Preparation of Standard Curve
    1. Prepare separate stock solutions of canthaxanthin (99%, CaroteNature, Lupsingen, Switzerland) and astaxanthin (99%, Acros Organics, Morris Plains, NJ) by diluting 25 mg of each standard to 50 mL with N,N-dimethylformamide.
    2. Mixed working standards ranging from 0.5 – 50 ppm prepared by stepwise dilution with methanol.

C. HPLC Analysis
    1. HPLC System: Hewlett Packard 1050 with autosampler, diode array detector, gradient pump and Chemstation software.
    2. Column: YMC carotenoid, 5 micron particle size, 4.6 x 250 mm.

3. Mobile phase: 99.5% HPLC grade methanol, 0.5% HPLC grade water with 50 ul trifluoroacetic acid.
4. Flow rate: 1.5 mL/min.
5. Wavelength: 470 nm
6. Elution time(s): astaxanthin – 10 min, canthaxanthin – 19 min.

Results

A summation of the results is presented in the attached table. Five of the eleven areas sampled produced urchins with detectable levels of astaxanthin, ranging in concentration from 1.4 to 7.1 parts per million (ppm) or ug/g. Ten of the eleven areas had urchins with canthaxanthin concentrations ranging from 5.7 to 7.85 ppm. One hundred percent of the animals from the 5 M Johnson Cove Station and 30M outside South Bay Station tested positive for canthaxanthin. It is of interest to note that all of the astaxanthin and canthaxanthin positive urchins showed little variability in concentration of either carotenoid.

Minimum levels of quantification (LOQ) for astaxanthnin and canthaxanthin were 0.5 and 1.0 ppm, respectively. The author of the method followed for this study lists the detection limit for canthaxanthin as 0.1 ppm. This ten-fold difference in sensitivity can be explained by the different detectors used by the respective HPLC systems. The UMaine group used an older photodiode array detector (PAD), which is not as sensitive as the ultraviolet (UV) detector used by Japan. Any future work done with urchins in the UMaine Chemical Food Safety Laboratory will employ a newer and more sensitive UV detector. It may also be fruitful to saponify several canthaxanthin-positive urchins to ensure that all of the canthaxanthin present in the animals is in the “free” form.

Batch (with station name, distance, depth, and date)	Number of Astaxanthin positive from 10 sea urchins tested	Number of Canthaxanthin positive from 10 sea urchins tested	Average level of Astaxanthin (µg/g, ppm)	Average level of Canthaxanthin (µg/g, ppm)
South Bay Station 5 M (edge), 100 ft, 11-7-07	0	2	0 ± 0	6.04 ± 0.49
Birch Point Outside 60-70M, 50 ft, 11-7-07	2	3	1.60 ± 0.15	7.07 ± 1.81
Birch Point Station 30 M, 50 ft, 11-7-07	0	1	0 ± 0	6.38 ± 0
Johnson Cove Station 30 M, 45 ft, 11-7-07	0	5	0 ± 0	6.60 ± 0.84
Birch Point Station Ø M (under), 50 ft, 11-7-07	0	0	0 ± 0	0 ± 0
Johnson Cove 120 M, 28 ft, 11-7-07	1 (from 4 sea urchins)	2 (from 4 sea urchins)	1.56 ± 0	7.85 ± 2.23
Johnson Cove Station 5 M (edge) 48 ft, 11-7-07	1	10	7.08 ± 0	6.11 ± 0.25
Johnson Cove Station Ø M (under), 48 ft, 11-7-07	10 (from 17 sea urchins)	17 (from 17 sea urchins)	1.44 ± 0.02	6.75 ± 0.53
Birch Point Station 5 M (edge), 50 ft, 11-7-07	0	3	0 ± 0	5.70 ± 0.03
South Bay Station Ø M (under), 100 ft, 11-7-07	1	7	1.44 ± 0	5.89 ± 0.63
South Bay Station 30M, 100 ft 11-7-07	0 (from 1 sea urchin)	1 (from 1 sea urchin)	0 ± 0	5.86 ± 0

Appendix A

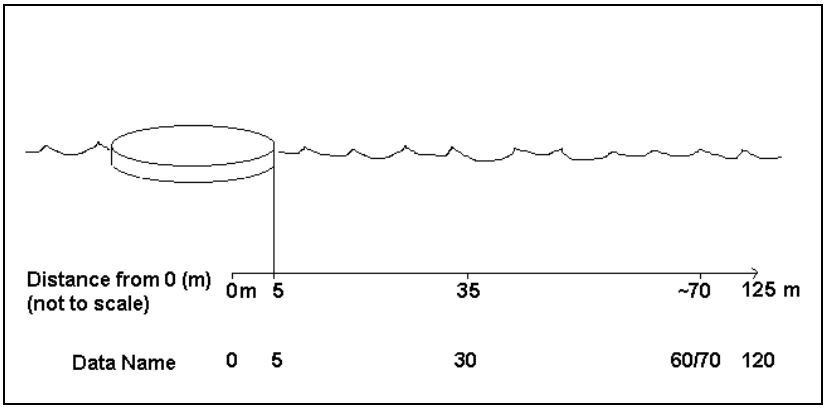
Project Notes

by Margaret Hunter, Maine Dept. of Marine Resources (DMR).

The sea urchins for this project were collected by DMR divers Jon Lewis and Robert Russell, tended by Chris Bartlett, from under commercial salmon aquaculture pens in Cobscook Bay, Maine, on November 7, 2007, after receiving a report of unacceptable levels of canthaxanthin in Maine sea urchins exported to Japan the previous winter.

Three salmon pens were sampled, at South Bay, Johnson Cove, and Birch Point. All pens were active or recently active. For more information about these lease areas, visit http://www.maine.gov/dmr/aquaculture/leaseinventory/cobscookbay.htm.

Divers went down a line from the edge of each pen to the ocean bottom, then swam in under the pen about 5 meters, then back out along a transect. The "zero" station (Ø) is under the pen, the 5m station is at the edge, the 30m station is 30m from the edge, etc.



The urchins were transported in a cooler to the University of Maine Food Chemical Safety Lab the next day.

The methodology modified for the analysis was translated from Japan's Ministry of Health, Labour, and Welfare, Department of Food Safety web page at http://www.mhlw.go.jp/topics/bukyoku/iyaku/syoku-anzen/zanryu3/2-042.html.

For making this project possible, our thanks go to the following:

- Jon Lewis and Robert Russell (DMR) – divers
- Chris Bartlett, Maine Marine Extension Team – boat, tending, photos
- Jennifer Robinson, salmon companies, and Jon Lewis (DMR) – coordination
- Dr. Brian Perkins and staff, U. Maine Food Chemical Safety Lab – analysis and report
- Minoru Kanaiwa – translations of Japanese documents