REFERENCES

Akiyama, D. M., Coelho, S. R., Lawrence, A. L., and Robinson, E. H. 1988. Apparent digestibility of feedstuffs by the marine shrimp, Penaeus vannamei Boone. Bull. Jap. Soc. Sci. Fish. 55(1) : 91.

_____. and Dominy, W. G. 1992. Penaeid shrimp nutrition for the commercial feed industry. Texas Shrimp Farming Manual. Vol1: Grow-out Technology Technical Report of Texas Agricultural Extension Service and Texas A&M University Sea Grant College Program, U.S.A. 50pp.

Andrews, A. G. and Starr, M. P. 1976. (3R, 3'R)-Astaxanthin from the yeast Phaffia rhodozyma. Phytochemistry 15: 1009.

Anonymous. 1988. Shrimp hatcheries. Coastal Aquaculture 1(1) : 4-6.

Association of Official Analytical Chemists. 1980. Official method of analysis. 13th ed. Washington D.C.: Association of Official Analytical Chemists.

Bidigare, R., Ondrusek, M., Kennicutt, M. C., Iturriaga, R., Harvey, H. R., Hoham, R. W., and Macko, S. A. 1993. Evidence for a photoprotective function for secondary carotenoids of snow algae. Phycol. 29: 427-434.

Bjerkeng, B., Storebakken, T. and Jensen, S.L. 1990. Response to carotenoids by rainbow trout in the sea: resorption and metabolism of dietary astaxanthin and canthaxanthin.

Aquaculture 91: 153-162.

Bold, H.C. and Wynne, M.J. 1978. Introduction to the Algae: Structure and Reproduction. New Delhi: Prentice-Hall of India Private Ltd.

Boonyaratpalin, M., Wannagowat, J., Borisut, C., and Boonchuay, S. 1994. Effect of various dietary canthaxanthin and astaxanthin levels on pigmentation of giant tiger shrimp. Techical paper no.18/1994. National Institute of Coastal Aquaculture, Songkhla.

Boussiba, S., and Vonshak, A. 1991. Astaxanthin accumulation in the green alga Haematococcus pluvialis. Plant Cell Physiol. 32(7) : 1077-1082.

Brown, M. R., Jeffrey, S. W., and Garland, C.D. 1991. Nutritional aspects Of microalgae used in mariculture; a Literature Review. Fishing Industry Research and Development Trust Accout 86/81. Department of Primary Industry, Canberra, ACT.

Bubrick, P. 1991. Production of astaxanthin from Haematococcus. Bioresource Technology 38: 237-239.

Catacutan, M., and Cruz D. M. 1989. Growth and midgut cells profile of Penaeus monodon juveniles fed water-soluble vitamin-deficient diets. Aquaculture 81: 137-144.

Chapman, V.J. 1962. The Algae. New Zealand: St. Martin's Press MacMillan.

Chindamaikul, T., and Phimonchinda, T. 1990. The Studies on comparison of nursing Penaeus monodon Fabricius with different feed.

Technical paper No. 34/1990, November. Phuket Coastal Aquaculture, Department of Fisheries.

Chu, F. E., Webb, K. L., Hepworth, D. A., and Casey, B. B. 1987. Metamorphosis of larvae of Crassostrea virginica fed microencapsulated diets. Aquaculture 64: 185-197.

Choubert, G., and Heinrich, O. 1993. Carotenoids pigment of the green alga Haematococcus pluvialis: assay on rainbow trout, Oncorhynchus mykiss, pigmentation in comparison with synthetic astaxanthin and canthaxanthin. Aquaculture 112: 217-226.

Cohen, Z. 1986. Products from microalgae. Handbook of Microalgal Mass Culture. A. Richmond ed. Florida : CRC Press, pp. 421-454.

Davies, B. H. 1976. Carotenoids. Chemistry and Biochemistry of Plants Pigments. 2 nd ed, vol 2. London: Academic Press.

Davies, J. B., and Weedon, B. C. L. 1960. Total synthesis of astacene. Proc. Chem. Soc. 182.

Droop, M. R. 1955. Carotenogenesis in Haematococcus pluvialis. Nature 175: 42.

Fan, L., Vonshak, A., and Boussiba, S. 1994. Effect of temperature and irradiance on growth of Haematococcus pluvialis (Chlorophyceae). Phycol. 30: 829-833.

Foss, P., Storebakken, T., Schieldt, K., Liaaen-Jensen, S., Austreng, E., and Streiff, K. 1984. Carotenoids in diets for salmonids. I Pigmentation with the individual optical isomers of astaxanthin in

comparison with canthaxanthin. Aquaculture 41:213-226.

Foss, P., Storebakken, T., Austreng, E., and Liaaen-Jensen, S. 1987. Carotenoids in diets for salmonids. V. Pigmentation of rainbow trout and sea trout with astaxanthin and astaxanthin dipalmitate in comparison with canthaxanthin. Aquaculture 65: 293-305.

Ghidalia, W. 1985. Structural and Biological Aspects of Pigment. In D. E. bliss and L.H. mentel (Editors). The Biology of Crustacea. vol 9, Integument, Pigment and Hormonal Processes. London: Academic Press.

Goodwin, T. W., and Jamikorn, M. 1954. Studies in carotenogenesis. II. Carotenoid synthesis in the alga Haematococcus pluvialis. Biochem. 57: 376.

Guillou, A., Khalil, M., and Adambounou, L. 1995. Effects of silage preservation on astaxanthin forms and fatty acid profiles of processed shrimp (Pandalus borealis) waste. Aquaculture 130: 351-360.

Harker, M. 1995. Carotenogenesis in the Chlorophyta Ph.D.dissertation, Liverpool John Moores University, UK.

Herring, P. 1969. Pigmentation and carotenoid metabolism of the marine isopod Idotea metallica. Mar. Bio. Ass. UK. 49: 767-779.

Johnson, E.A., and An, G.H. 1991. Astaxanthin from microbial sources. Critical Reviews in Biotechnology 11(4): 297-326.

Jones, D.A., Kurmaly, K., and Arshard. 1987. Penaeid shrimp hatchery trials using microencapsulated diets. Aquaculture 64: 133-142.

Kakizono, T., Kobayashi, M., and Nagai S. 1992. Effect of carbon/nitroge ratio on encystment accompanied with astaxanthin formation in a green alga, Haematococcus pluvialis. Fermentation and Bioengineering 74(6): 403-405.

Katayama, T., Hirata, K., and Chichester, C. O. 1971. The Biosynthesis of astaxanthin - IV. The Carotenoids in the prawn, Penaeus japonicus Bate (Part1). Bull. Jpn Soc. Sci. Fish. 37(7): 614-620.

Kobayashi, M., Kakizono, T., and Nagai, S. 1991. Astaxanthin production by a green alga Haematococcus pluvialis Accompanied with Morphological changes in Acetate Media. Fermentation and Bioengineering 71(5): 335-339.

_____., Kakizono, T., Nishio, N., and Nagai, S. 1992A. Effects of light Intensity, light quality and illumination cycle on astaxanthin formation in a green alga, Haematococcus pluvialis. Fermentation and Bioengineering 74(1): 61-63.

_____., Kakizono, T., Yamagushi, K., Nishiro, N., and Nagai, N. 1992B. Growth and astaxanthin formation of Haematococcus pluvialis in heterotrophic and mixotrophic conditions. Fermentation and Bioengineering 74(1): 17-20.

Kvalheim, B., and Knutsen, G. 1985. Pigmentation of microalgae. Plant Sci. 41:169.

Laing, I. 1987. The use of artificials in rearing bivalve spat. Aquaculture 65: 243-249.

Latscha, T. 1989. The role of astaxanthin in shrimp pigmentation. Advances in Tropical Aquaculture 319-325.

_____. 1990. Carotenoids in Aquatic animal Nutrition. Roche publication No. 2175 (in press) 68-79.

Lavens, P., Sorgeloos, P., Dhert, P., and Devresse, B. 1992. Latest developments in larval food production. Aquaculture and Fisheries Management (in press).

Lee, Y. K., and Ding, S.Y. 1992. Can Haematococcus lacustris (Chlorophyta) recovered from photoinhibition of photosynthesis? Plant Cell Physiol. 33(6) : 815-818.

Lee, Y. K., and Soh, C.W. 1991. Accumulation of astaxanthin in Haematococcus lacustris. Phycol. 27: 575-577.

Menasveta, P., Worawattanamateekul, W., Latscha, T., and Clark, J. S. 1993. Correction of black tiger prawn (Penaeus monodon) colouration by astaxanthin. Aquacultural Engineering 12: 203-213.

Motoh, H. 1979. Larvae of Decapod Crustacea of the Philippines III. Larval development of the giant tiger prawn, Penaeus monodon reared in the labolatory. Bull. Jpn Soc. Sci. Fish. 45 (10): 1201-1216.

_____. 1984. Biology and ecology of Penaeus monodon. Proceeding of the first international conference on the culture of penaeid prawn/shrimp. The Philippines.

Negre-Sadargues, G., Castillo, R., Petit, H., Sance, S., Martinez, R. G.,

Milicua, J. C. G., Choubert, G., and Trilles, J. P. 1993. Utilization of synthetic carotenoids by the prawn Penaeus japonicus reared under laboratory conditions. Aquaculture 110:151-159.

Powtongsook, S. 1993. Strain selection and culture of Dunaliella salina (Chlorophyceae) for beta-carotene production. Master's Thesis, Chulalongkorn University.

Pringsheim, E. G. 1966. Nutritional requirement of Haematococcus pluvialis and related species. Phycol. 2: 1.

Roche News. 1993. Roche Aquaculture Center. Vol. 1/1993.

SAS, 1985. The Statistic Analysis System, SAS Institute Inc. USA., 95 pp.

Shiedt, K., Bischof, S., and Gling, E. 1993. Metabolism of carotenoids and in vivo racemization of (3S, 3'S)-astaxanthin in the crustacean penaeus. Methods in Enzymology vol 14, Academic Press Inc.

Smith, G. M. 1950. Freshwater Algae of the United States. 2 nd ed. pp. 109-111. California: McGraw Hill Book Co.

Spencer, K. 1989. Pigmentation supplements for animal feed composition. US Patent No. 4,871,551.

Stanier, R. Y., Kunizawa, M. M., and Cohen-Bazire, G. 1971. Purification and property of unicellular blue-green algae (Order Chroococales). Bacterial Rew. 35: 171-201.

Storebakken, T., and No Kyoon, H. 1992. Pigmentation of rainbow trout. Aquaculture 100: 209-229.

Strickland, J. D. H., and Parsons, T. R. 1977. A Practical Handbook of Seawater Analysis. Second ed. Bulletin 167, Fisheries Research board of Canada, Ottawa, 284 pp.

Sutjaritvongsanon, S. 1984. Rearing of the larvae stages of prawn, Penaeus japonicus Bate, using micro-particulated Diets. Thai Fisheries GaZette. 37(3): 243-248.

Tanaka, Y., Malsuguchi, H., Katayama, T., Simpson, K. L., and Chichester, C. O. 1975. The Biosynthesis of astaxanthin-XVIII. The metabolism of the carotenoids in the prawn Penaeus japonicus Bate. Bull. Jpn Soc. Sci. Fish. 42 (2): 197-202.

Terao, J. 1989. antioxidant activity of β-carotene-related carotenoids in solution. Lipids 24: 659.

Teshima, S., and Kanazawa, A. 1984. Effects of protein, lipid and carbohydrate levels in purified diets on growth and survival rates of the prawn larvae. Bull. Jpn Soc. Sci. Fish. 50 (10): 1709-1715.

Tjahjono, A. E., Kakizono, T., Hayama, Y., Nishio, N., and Nagai, S. 1994. Isolation of resistant mutants against carotenoid biosynthesis inhibitors for green alga Haematococcus pluvialis, and their hybrid formation by protoplast fusion for breeding of higher astaxanthin Producers. Fermentation and Bioengineering 77(4): 352-357.

Thamrujikul, W. 1990. Production of feed for larvae of giant tiger prawn (Penaeus monodon Fabricius). Master's Thesis,

Chulalongkorn University.

Torrissen, O. J. 1984. Pigmentation of salmonids: Effect of carotenoids in eggs and start-feeding diet on survival and growth rate. Aquaculture 43: 185-193.

Weber, S. 1990. Determination of added stabilized astaxanthin in fish feeds and premix with HPLC. Roche-publication, Index No. 2101, 59-61.

Yamada, S., Tanaka, Y., Sameshima, M., and Ito, Y. 1990. Pigmentation of prawn (Penaeus japonicus) with carotenoids I. Effect of Dietary astaxanthin, β-carotene and canthaxanthin on pigmentation. Aquaculture 87: 323-330.

Yong, Y. Y. R., and Lee, Y. K. 1991. Do carotenoids play a photoprotective role in the cytoplasm of Haematococcus lacustris (Chlorophyta) ? Phycologia 30 (3) : 257-261.

Appendix 1 Modified Bold's basal medium (Spencer,1989)

Sodium chloride	12.5	mg/l
Calcium chloride	12.5	mg/l
Magnesium sulphate	38.0	mg/l
Potassium sulphate dibasic	93.0	mg/l
Potassium sulphate monobasic	44.0	mg/l
Ethylene diamine tetraacetic acid (EDTA)	25.0	mg/l
Ferric chloride	2.5	mg/l
Sodium molybdate	0.35	mg/l
Zinc sulphate	4.4	mg/l
Manganese chloride	0.73	mg/l
Copper sulphate	0.77	mg/l
Cobalt choride	0.23	mg/l
Thiamin	4.1	mg/l
Sodium acetate	1.4	g/l
Urea	0.12	g/l

Appendix 2 Chlorophylls and total carotenoids determination

(Strickland & Parsons, 1977)

Pretreatment :

1. Precombust Millipore filter at 100 ^{o}C for 1 hr.
2. Store filters in vacuum desiccator over $KMnO_4$ crystals until use.

Chlorophylls and total carotenoids determination

1. Filter sample of algae under suction.
2. Place the filter in a 15 ml stoppered centrifuge tube. Add proximated 8 ml of 90% acetone, stopper the tube and dissolve the filter by shaking the tube vigorously.
3. Allow the pigments to be extracted by placing the tube in a refrigerator in complete darkness for about 20 hr.
4. Cool to the room temperature. Add 90% acetone to make the extracts up to exactly 10 ml.
5. Centrifuge the tubes for 5-10 ml. Decant the supernatant and measure at 665 nm, 645 nm and 630 nm.

$$C \text{ (chlorophyll a)} = 11.6\, E_{6650} - 1.31\, E_{6450} - 0.14\, E_{6300}$$

$$C \text{ (chlorophyll b)} = 20.7\, E_{6450} - 4.34\, E_{6650} - 4.42\, E_{6300}$$

$$C \text{ (chlorophyll c)} = 55\, E_{6300} - 4.64\, E_{6650} - 16.3\, E_{6450}$$

$$C \text{ (total carotenoids)} = 4.0\, E_{4800}$$

6. Calculate the concentration of pigments from the equation

mg pigment / m^3 (μg/l) = C/V (V=volume of filtered sample in litre)

Appendix 3 Ash free dry weight (AFDW) determination

(Powtongsook, S. 1993)

Pretreatment

1. Precombust Whatman GF/C filters at 100% for 1 hr.
2. Store filters im vacuum desiccator over $KMnO_4$ crystals until use.

Dry weight determination

1. Carefully weight precombusted filters to 4 decimal places.
2. Place filters in filter unit and filter culture until filter appears completely dry.
3. Remove filter from filter unit and dry at 100 ^{o}C for 1h and then place in vacuum desiccator over $KMnO_4$ overnight.
4. Weight dried filter containing algae to 4 decimal places.

Dry weight = (weight of filter plus algae) - (weight of filter)

Ash-free dry weight (organic dry weight) determination

1. Take filters from above dry weight determination and ash at 450 ^{o}C for 5 hr.
2. Cool filters in a vacuum desiccator over $KMnO_4$.
3. Rapidly and carefully weight filters.

Ash-free dry weight = dry weight - weight after ashing

Appendix 4 Ash determination (AOAC, 1980)

Apparatus : Muffle furnace (Carbolite, model EML 11/2 serial no. 11/86/1468, Bandford, Sheffield, England).

Method :

1. Weigh 2 g of dry sample into porcelain crucible and place in temperature controlled furnace preheated to 600 ^{o}C .
2. Hold at this temperature 2 hr.
3. Transfer crucible directly to desiccator, cool and weigh immediately.

% ash = (weight of remained ash /weight of dry sample) x 100

Appendix 5 Fiber determination (AOAC, 1980)

Reagent : 1. 0.255 N sulfuric acid solution

2. 0.313 N sodium hydroxide solution

3. 95% ethyl alcohol

Apparatus : 1. Crude fiber digestion apparatus (model RF-16/6 composed of hot plate, beaker 600 ml and round condenser)

2. Filtered fibre No. 41

3. Crusible

4. Muffle furnace

Method :

1. Boiled sulfuric acid solution 200 ml in 600 ml beaker and placed on hot plate.

2. Poured the weight sample (out of fat) into the beaker and digested until homogeneous (during the digestion, should be maintained the volume of sulfuric acid by covering the beaker with condensor).

3. Filtered the digested sample with filtered paper (dry exactly at 105 ^{o}C 2 hr) and washed the residue with distilled water until the digested was neutral.

4. Filled the residue into the beaker and added NaOH 200 ml.

5. Boiled the extract for 30 min.

6. Filtered the digest with the same filtered paper and washed the residue with distilled water until the digested was neutral.

7. Washed the residue again with 95% ethanol 30 ml then put the filtered paper in the crusible and dried at 100 ^{o}C for 2 hr.

8. Transferred crucible directly to desiccator, cool and weigh.

9. Placed the crusible in temperature controlled furnace preheated to 600 ^{o}C for 3 hr.

10. Transferred crucible directly to desiccator, cool and weigh again.

% Fiber = (weight of dry residue + weight of dry filtered paper) - (weight of filtered paper before used - %ash)/ weight of sample

Appendix 6 **Moisture content determination** (AOAC, 1980)

Apparatus : Sartorius Thermo-control model L310

Method :

1. Weigh 2 g of sample and placed in the dried extractly tray.
2. Put the tray in the hood part of the Sartorius Thermo-control equipment which controlled temperature of infrared ray at 130 ^{o}C.
3. Read the moisture content (%) of the sample when it showed the signal.

Appendix 7 Fat determination (AOAC, 1980)

Apparatus : Soxtherm automatic S-11, Garhardt.

Method :

1. Weigh 2 g of sample and covered with dry Whatman No.1 filter.
2. Put the cover into the thimble which placed in the accurately weighed soxhlet bottle then added petrolium ether 80 ml into the bottle.
3. Set the Soxtherm automatic which controlled silicone oil at 150 °C.
4. Left for the extraction for 4-6 hr then evaporated petrolium ether from the extract.
5. Dried the bottle at 100 °C for 1 hr and transferred to the desiccator.
6. Weigh and calculated according to the follows:

Fat content (%) = (weight of extracted fat x 100)/weight of sample

Appendix 8 Protein determination (AOAC, 1980)

Reagents : 1. Conc. Sulfuric acid solution

2. 0.1 N Sulfuric acid solution

3. 50% NaOH solution

4. 4% Boric acid solution

5. Catalyst (K_2SO_4 : Se = 1000 : 1)

6. Indicator (Methyl Red + Methylene Blue)

Apparatus : Gerhardt Kjeldatherm Digestion Unit

Gerhardt Vapodest1

Method :

1. Weighed 2 g of dry sample and put in a digestive tube.
2. Added 1 tablet of catalyst.
3. Set the Kjeldatherm and digested until the sample became black.
4. Digested completely for 4 hr (set the beginning temperature at 100 °C and increased 20 °C every 15-20 min until controlled at 380 °C).
5. Cooled until room temperature then added 90 ml distilled water.
6. Set the Vapodest 1 for distilling the digested sample, added 50% NaOH and kept the distillated in boric acid which added indicator 5-6 drops.
7. Titrated the solution with 0.5 N sulfuric acid.

Protein content (%) = (A x B x 6.25 x 1.4)/ C

A = normality of sulfuric acid used in titration

B = volume of sulfuric acid used in titration (ml)

C = weight of sample (g)

Appendix 9 Statistical Analysis

Survival of zoea stage

General Linear Models Procedure

Dependent Variable: SURVIVAL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1245.450000	177.921429	1.96	0.1460
Error	12	1089.500000	90.791667		
Corrected Total	19	2334.950000			

R-Square	C.V.	Root MSE	SURVIVAL Mean
0.533395	14.42614	9.528466	66.0500000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	1129.750000	376.583333	4.15	0.0312
P	4	115.700000	28.925000	0.32	0.8601

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	1129.750000	376.583333	4.15	0.0312
REP	4	115.700000	28.925000	0.32	0.8601

Duncan's Multiple Range Test for variable: SURVIVAL

A!pha= 0.05 df= 12 MSE= 90.79167

Number of Means 2 3 4

Critical Range 13.11 13.73 14.14

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TREAT
	A	76.000	5	Algal astaxanthin-added diet
	A			
B	A	68.600	5	Control diet
B	A			
B	A	64.400	5	Synthetic astaxanthin-added diet
B				
B		55.200	5	Natural food

Appendix 9 (continue)

Survival of mysis stage

General Linear Models Procedure

Dependent Variable: SURVIVAL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1058.280292	211.656058	15.66	0.0098
Error	4	54.055908	13.513977		
Corrected Total	9	1112.336200			

R-Square	C.V.	Root MSE	SURVIVAL Mean
0.951403	5.486770	3.676136	67.0000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	648.6824167	216.2274722	16.00	0.0108
REP	2	409.5978750	204.7989375	15.15	0.0136

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	260.8042417	86.9347472	6.43	0.0520
REP	2	409.5978750	204.7989375	15.15	0.0136

Duncan's Multiple Range Test for variable: SURVIVAL

Alpha= 0.05 df= 4 MSE= 13.51398

WARNING: Cell sizes are not equal.

Harmonic Mean of cell sizes= 2.4

Number of Means 2 3 4

Critical Range 9.334 9.532 9.567

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	76.667	3	Natural food
A			
A	69.723	3	Algal astaxanthin-added diet
B	58.125	2	Synthetic astaxanthin-added diet
B	57.290	2	Control diet

Appendix 9 (continue)

Survival of postlarval stage

General Linear Models Procedure

Dependent Variable: SURVIVAL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1245.450000	177.921429	1.96	0.1460
Error	12	1089.500000	90.791667		
Corrected Total	19	2334.950000			

R-Square	C.V.	Root MSE	SURVIVAL Mean
0.533395	14.42614	9.528466	66.0500000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	1129.750000	376.583333	4.15	0.0312
P	4	115.700000	28.925000	0.32	0.8601

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	1129.750000	376.583333	4.15	0.0312
REP	4	115.700000	28.925000	0.32	0.8601

Duncan's Multiple Range Test for variable: SURVIVAL

Alpha= 0.05 df= 12 MSE= 90.79167

Number of Means 2 3 4

Critical Range 13.11 13.73 14.14

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	76.000	5	Algal astaxanthin-added diet
A			
B A	68.600	5	Control diet
B A			
B A	64.400	5	Synthetic astaxanthin-added diet
B	55.200	5	Natural food

Appendix 9 (continue)

Length of postlarva 15 fed different diets.

General Linear Models Procedure

Dependent Variable: Length

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.06100764	0.00871538	1.65	0.1234
Error	192	1.01358752	0.00527910		
Corrected Total	199	1.07459516			

R-Square	C.V.	Root MSE	LL Mean
0.056773	7.416227	0.072657	0.97970874

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	0.04382128	0.01460709	2.77	0.0431
REP	4	0.01718635	0.00429659	0.81	0.5177

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	0.04382128	0.01460709	2.77	0.0431
REP	4	0.01718635	0.00429659	0.81	0.5177

Duncan's Multiple Range Test for variable: Length

Alpha= 0.05 df= 192 MSE= 0.005279

Number of Means 2 3 4

Critical Range .0289 .0304 .0313

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	1.0019	50	Algal astaxanthin-added diet
A			
B A	0.9843	50	Natural food
B			
B	0.9674	50	Control diet
B			
B	0.9652	50	Synthetic astaxanthin-added diet

TREAT=1 : Larvae fed Natural diet

N Obs	Variable	N	Minimum	Maximum	Mean	StdDev
50	REP	50	1.0000000	5.0000000	3.0000000	1.4285714
	LENGTH	50	8.2000000	11.5000000	10.1380000	0.7537363
	LL	50	0.7961165	1.1165049	0.9842718	0.0731783

TREAT=2 : Larvae fed Control diet

N Obs	Variable	N	Minimum	Maximum	Mean	Std Dev
50	REP	50	1.0000000	5.0000000	3.0000000	1.4285714
	LENGTH	50	7.8000000	11.3000000	9.9640000	0.7628518
	LL	50	0.7572816	1.0970874	0.9673786	0.0740633

TREAT=3 : Larvae fed Algal astaxanthin-added diet

N Obs	Variable	N	Minimum	Maximum	Mean	Std Dev
50	REP	50	1.0000000	5.0000000	3.0000000	1.4285714
	LENGTH	50	8.8000000	12.0000000	10.3200000	0.6954664
	LL	50	0.8543689	1.1650485	1.0019417	0.0675210

TREAT=4 : Larvae fed Synthetic astaxanthin-added diet

N Obs	Variable	N	Minimum	Maximum	Mean	Std Dev
50	REP	50	1.0000000	5.0000000	3.0000000	1.4285714
	LENGTH	50	8.1000000	11.5000000	9.9420000	0.7733020
	LL	50	0.7864078	1.1165049	0.9652427	0.0750779

Appendix 9 (continue)

Astaxanthin accumulated in postlarva 15 fed different diets.

General Linear Models Procedure

Dependent Variable: Concentration

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7983.954150	1995.988538	245.94	0.0004
Error	3	24.347050	8.115683		
Corrected Total	7	8008.301200			

R-Square	C.V.	Root MSE	CONC Mean
0.996960	2.240947	2.848804	127.125000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	7964.297700	2654.765900	327.12	0.0003
REP	1	19.656450	19.656450	2.42	0.2175

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	7964.297700	2654.765900	327.12	0.0003
REP	1	19.656450	19.656450	2.42	0.217

Duncan's Multiple Range Test for variable: Concentration

NOTE: This test controls the type I comparisonwise error rate, notthe experimentwise error rate

Alpha= 0.05 df= 3 MSE= 8.115683

Number of Means 2 3 4

Critical Range 9.054 9.090 9.090

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	179.540	2	Natural food
B	122.565	2	Algal astaxanthin-added diet
C	109.070	2	Synthetic astaxanthin-added diet
D	97.325	2	Control diet

Appendix 9 (continue)

Probit Analysis : Time for 50% cumulative mortality of low salinity stressed shrimp fed natural food.

Confidence Limits for Effective Day

95% Confidence Limits

Prob	TIME	Lower	Upper
.01	1.79001	1.03096	2.71094
.02	2.47053	1.50078	3.59659
.03	3.03091	1.90424	4.30376
.04	3.53478	2.27756	4.92643
.05	4.00580	2.63439	5.49917
.06	4.45584	2.98167	6.03917
.07	4.89186	3.32346	6.55643
.08	5.31835	3.66246	7.05735
.09	5.73842	4.00054	7.54638
.10	6.15437	4.33911	8.02672
.15	8.22263	6.07123	10.36798
.20	10.35176	7.92331	12.71584
.25	12.61270	9.94924	15.16062
.30	15.06100	12.19649	17.76872
.35	17.75203	14.71535	20.60443
.40	20.74885	17.56365	23.74130
.45	24.12900	20.81053	27.27269
.50	**27.99274**	**24.54036**	**31.32559**
.55	32.47517	28.85787	36.08155
.60	37.76562	33.89734	41.80958
.65	44.14106	39.84381	48.91798
.70	52.02799	46.98238	58.04094
.75	62.12733	55.80190	70.21094

.80	75.69665	67.21024	87.27167
.85	95.29717	83.06485	113.02446
.90	127.32307	107.92566	157.21622
.91	136.55201	114.91234	170.34775
.92	147.33770	122.99543	185.89035
.93	160.18300	132.51799	204.65750
.94	175.85743	144.00078	227.90782
.95	195.61480	158.28447	257.71764
.96	221.68123	176.84599	297.82675
.97	258.53378	202.61815	355.88691
.98	317.17636	242.69364	451.12177
.99	437.75857	322.34567	655.96259

Appendix 9 (continue)

Probit Analysis : Time for 50 % cumulative mortality of low salinity stressed shrimp fed control diet

Confidence Limits for Effective Day

95% Confidence Limits

Prob	TIME	Lower	Upper
.01	2.70562	1.74558	3.80088
.02	3.61715	2.43424	4.92019
.03	4.34883	3.00564	5.79646
.04	4.99526	3.52198	6.55762
.05	5.59132	4.00649	7.25037
.06	6.15437	4.47081	7.89779
.07	6.69452	4.92177	8.51323
.08	7.21827	5.36381	9.10522
.09	7.73010	5.79999	9.67965
.10	8.23325	6.23258	10.24073
.15	10.68953	8.39125	12.93699
.20	13.15453	10.62175	15.58783
.25	15.71764	12.99366	18.30309
.30	18.44233	15.56039	21.15789
.35	21.38709	18.37346	24.22026
.40	24.61503	21.48884	27.56427
.45	28.20106	24.97128	31.28048
.50	**32.23993**	**28.89866**	**35.48839**
.55	36.85724	33.36771	40.35407
.60	42.22678	38.50430	46.11682
.65	48.60003	44.48483	53.13079
.70	56.36019	51.58109	61.93622
.75	66.13038	60.25077	73.40228

.80	79.01564	71.32663	89.06241
.85	97.23657	86.48771	112.02471
.90	126.24581	109.80276	150.07767
.91	134.46318	116.26896	161.13026
.92	143.99755	123.70828	174.08660
.93	155.26323	132.41905	189.56666
.94	168.89024	142.85192	208.52135
.95	185.89768	155.73041	232.50391
.96	208.07991	172.31758	264.28014
.97	239.00972	195.10253	309.43181
.98	287.35687	230.04652	381.73898
.99	384.16783	298.09057	531.80814

Appendix 9 (continue)

Probit Analysis : Time for 50% cumulative mortality of low salinity stressed shrimp fed algal astaxanthin-added diet.

Confidence Limits for Effective day

95% Confidence Limits

Prob	TIME	Lower	Upper
.01	4.00473	2.62184	5.51815
.02	5.31534	3.64285	7.07962
.03	6.36127	4.48722	8.29389
.04	7.28165	5.24838	9.34401
.05	8.12767	5.96118	10.29659
.06	8.92478	6.64308	11.18448
.07	9.68775	7.30432	12.02667
.08	10.42609	7.95149	12.83530
.09	11.14633	8.58918	13.61870
.10	11.85321	9.22074	14.38287
.15	15.28969	12.35968	18.04460
.20	18.71846	15.58084	21.63600
.25	22.26670	18.97972	25.31624
.30	26.02284	22.62330	29.19836
.35	30.06684	26.56950	33.39011
.40	34.48382	30.87436	38.01413
.45	39.37395	35.59706	43.22371
.50	**44.86312**	**40.80787**	**49.21765**
.55	51.11754	46.60333	56.25698
.60	58.36649	53.13074	64.69051
.65	66.94084	60.62222	75.00607
.70	77.34358	69.44578	87.93649
.75	90.39057	80.20156	104.67858

.80	107.52489	93.93860	127.38474
.85	131.63775	112.72270	160.45885
.90	169.80202	141.50762	214.95054
.91	180.57058	149.46418	230.73050
.92	193.04452	158.60340	249.20828
.93	207.75715	169.28475	271.26053
.94	225.51823	182.05026	298.23093
.95	247.63551	197.76847	332.31256
.96	276.40725	217.95196	377.40717
.97	316.39922	245.57311	441.38087
.98	378.65889	287.72479	543.62744
.99	502.58087	369.19039	755.23158

Appendix 9 (continue)

Probit Analysis : Time for 50% cumulative mortality of low salinity stressed shrimp fed synthetic astaxanthin-added diet.

Confidence Limits for Effective Day

95% Confidence Limits

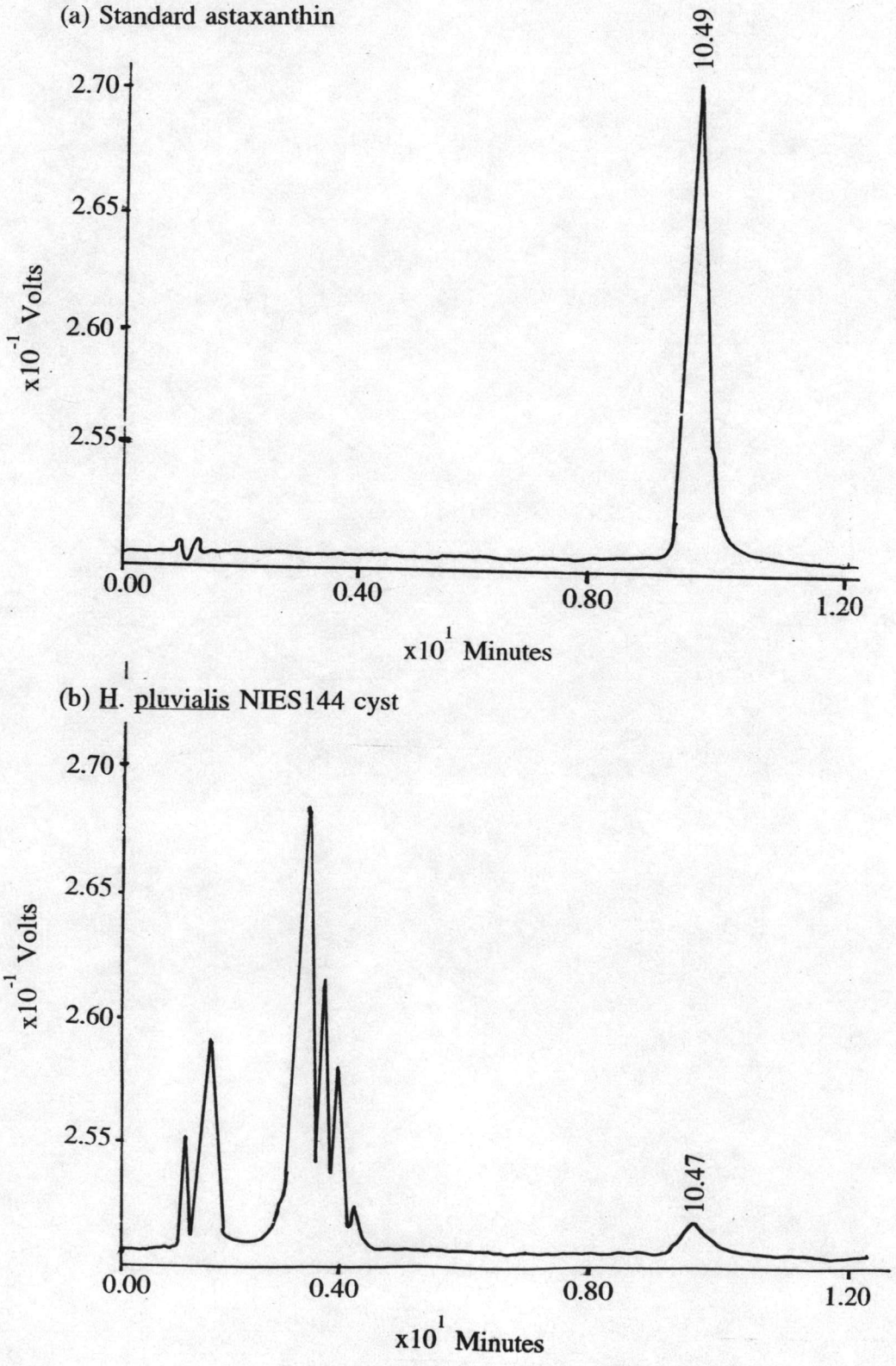
Prob	TIME	Lower	Upper
.01	2.18108	1.24016	3.30637
.02	3.03558	1.83153	4.40806
.03	3.74396	2.34515	5.29142
.04	4.38383	2.82407	6.07154
.05	4.98417	3.28460	6.79084
.06	5.55949	3.73499	7.47042
.07	6.11832	4.18014	8.12257
.08	6.66619	4.62327	8.75521
.09	7.20694	5.06666	9.37378
.10	7.74340	5.51199	9.98228
.15	10.42382	7.80701	12.96055
.20	13.20164	10.28366	15.96732
.25	16.16779	13.01002	19.12037
.30	19.39534	16.04605	22.51158
.35	22.95864	19.45348	26.23576
.40	26.94339	23.29952	30.40818
.45	31.45575	27.65869	35.18231
.50	**36.63377**	**32.61558**	**40.77221**
.55	42.66416	38.27424	47.48060
.60	49.80936	44.78328	55.73465
.65	58.45436	52.38326	66.14469
.70	69.19357	61.47890	79.63031
.75	83.00660	72.75966	97.70429

.80	101.65651	87.46079	123.13609
.85	128.74672	108.05818	161.73828
.90	173.31323	140.60546	228.58004
.91	186.21392	149.79018	248.57573
.92	201.31936	160.43245	272.31428
.93	219.34667	172.98974	301.07430
.94	241.39514	188.15715	336.84004
.95	269.25926	207.05799	382.89605
.96	306.13222	231.66821	445.18181
.97	358.45306	265.91731	535.90727
.98	442.10154	319.32620	685.93366
.99	615.30542	425.91321	1012.57470

Appendix 10 HPLC chromatograms of astaxanthin content.

(a) Standard astaxanthin

(b) H. pluvialis NIES144 cyst

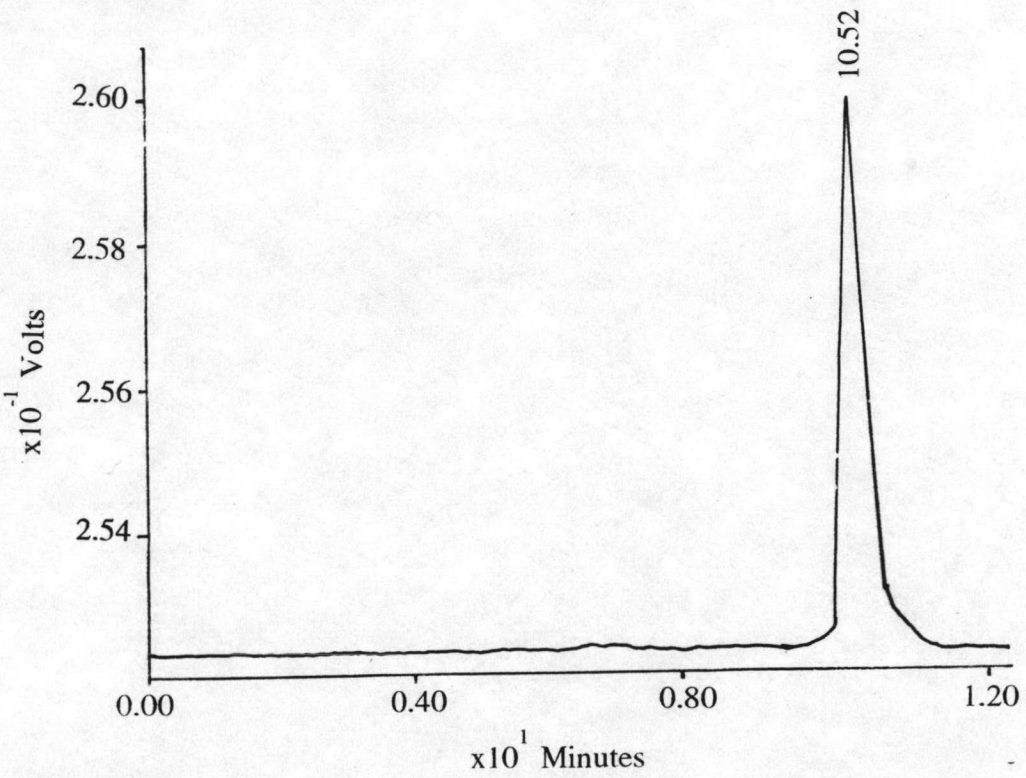


Appendix 10 (continue) HPLC chromatograms of astaxanthin content.

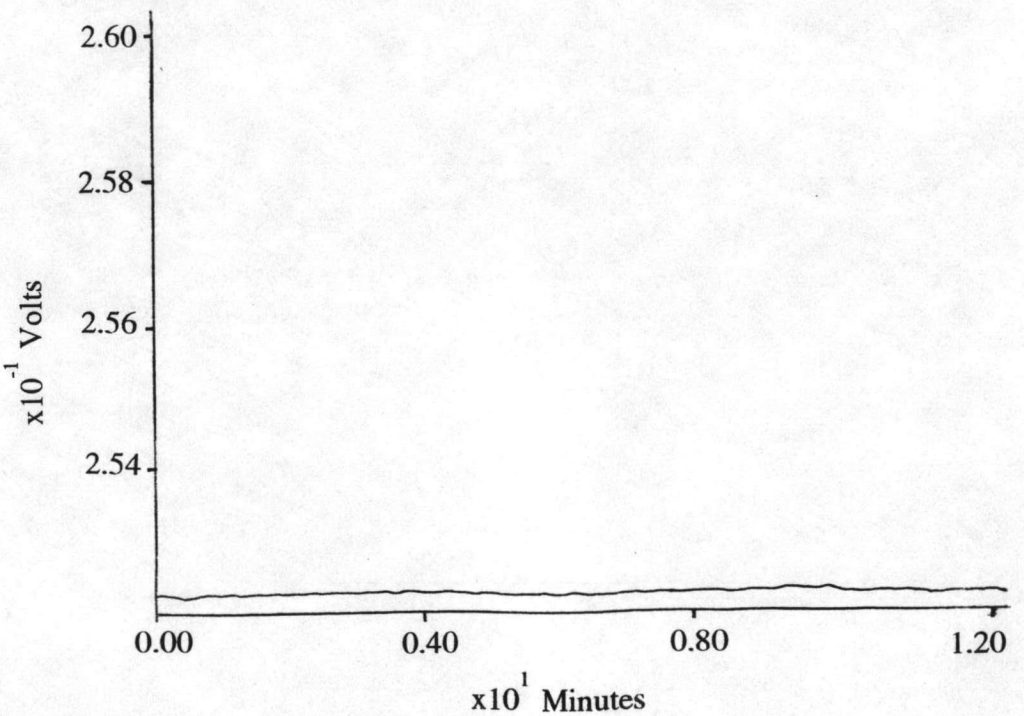
(c) synthetic astaxanthin

(d) control diet

(c) Synthetic astaxanthin



(d) Control diet

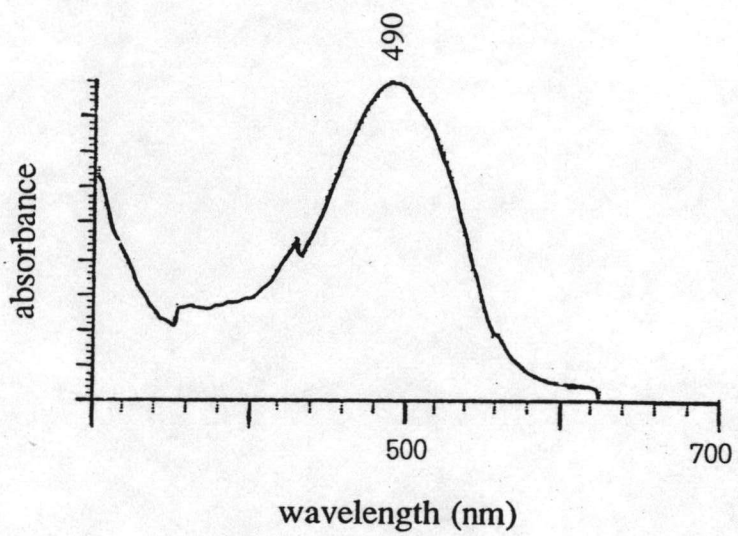


Appendix 11 Spectrum showing maximum absorption of astaxanthin

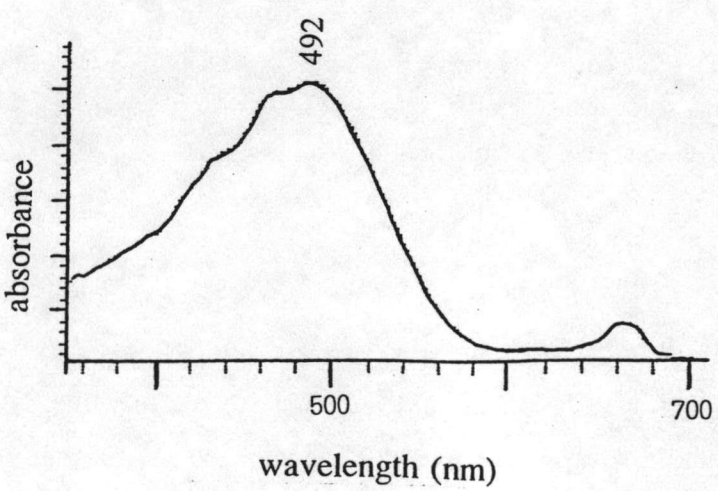
(a) Standard astaxanthin

(b) H. pluvialis NIES144 cyst

(a) Standard astaxanthin



(b) H. pluvialis NIES144 Cyst

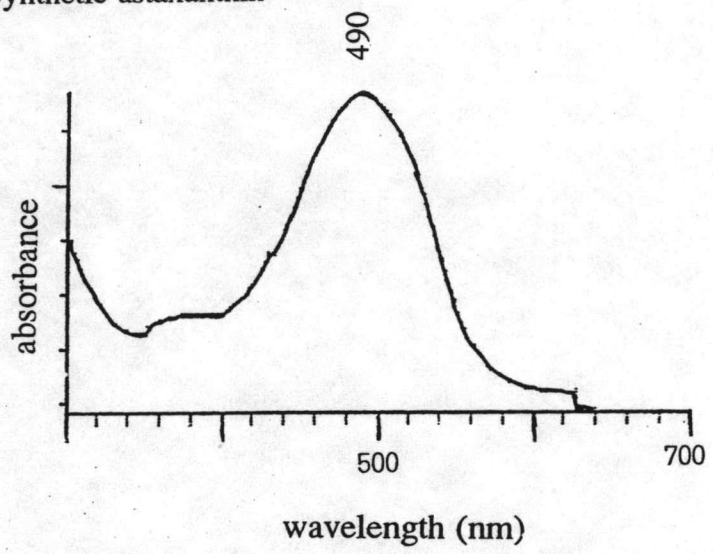


Appendix 11 (continue) Spectrum showing maximum absorption of astaxanthin

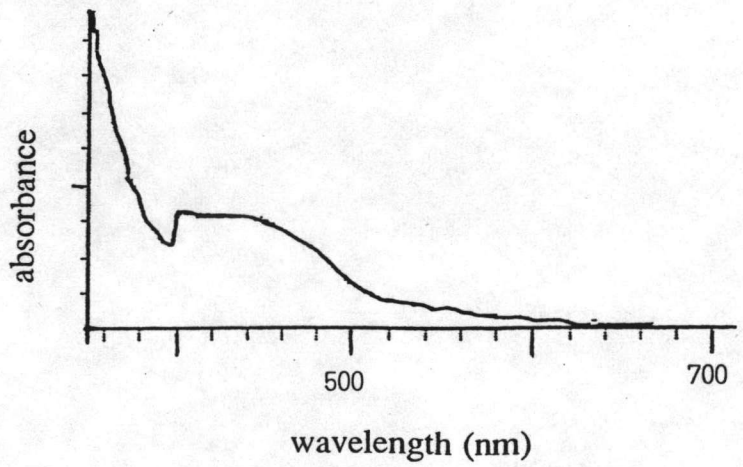
(c) synthetic astaxanthin

(d) control diet

(c) Synthetic astaxanthin



(d) control diet



Appendix 12 Calculation of astaxanthin concentration in H. pluvialis

At 492 nm, $E_{1\%}$ = 2,200 means substance 1 g dissolved in 100ml solvent (using DMSO: Dimethyl sulfoxide, as a solvent) was determined at wavelength 492 nm has absorbance 2,200 unit.

2,200 unit comes from substance = 1 g/100ml

1 unit _____ = 1/2,200 g/100ml

= 4.5×10^{-4} g/100ml

= 4.5×10^{-6} g/ml

= 4.5 µg/ml

So, Factor for calculation of astaxanthin concentration in H. pluvialis was 4.5

Example : Substance A has optical density at 492 nm = 0.862, volume of starting culture 11 ml and volume after extraction 5 ml.

Absorbance 1 unit comes from standard substance 4.5 µg/ml

_____ 0.862 unit _____ 4.5 x 0.862 µg/ml

= 3.879 µg/ml

hence, volume of extract = 5 ml

so, total content = 3.879 x 5 = 19.395 µg/5ml extract

= 19.395 µg/11ml culture

= 1.79 µg/ ml culture

= 1.79 mg/l culture

BIOGRAPHY

Miss Jintana Darachai was born on October 3, 1969 in Bangkok. She graduated with a Bachelor degree in Biology (B. Sc. Hons.) from Department of Science, Srinakharinwirot University, Bangkhen Campus in 1990. She began to study her Biotechnology class at Chulalongkorn University in 1993. After graduation, she will serve as an instructor at the Department of Marine Science, Faculty of Fisheries, Kasetsart University, Bangkok.