JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2014, Vol. 2, No. 2, pp:203-208
Available at www.jcrs010.com

ORIGINAL ARTICLE

IMPROVEMENT OF TOTAL POLYPHENOL CONTENT, TOTAL CAROTENOID AND ANTIOXIDANT ACTIVITIES OF *ARTEMIA SALINA* NAUPLII BIOENCAPSULATED WITH GREEN TEA EXTRACT

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ABSTRACT: The effect of green tea extract (GTE) bioencapsulation on total polyphenol content (TPC), total carotenoid (TC) and antioxidant activities of *Artemia salina* nauplii was investigated. Green tea powder was extracted with distilled water at 80°C for five (5) minutes and adjusted to 3.50% salinity with artificial seawater powder. Bioencapsulation was conducted using different naupliar stages of *Artemia* (newly hatched, Instar I and Instar II) for 6 hrs at thermo-controlled chamber set at 25°C with aeration and light support. Results showed that *Artemia* nauplii were able to accumulate polyphenols present in green tea into their body based on TPC and antioxidant capacities. Instar II nauplii enriched for 6 hours with GTE gave the highest TPC (20.08 ± 0.97 mg GAE g^{-1} $sample^{-1}$), TC (115.34 ± 4.86 mg g^{-1} $sample^{-1}$and antioxidant activities determined by DPPH free radical scavenging activity (73.04 ± 1.95 mg VCE g^{-1} $sample^{-1}$) and total antioxidant activity (18.08 ± 1.08 mg VCE g^{-1} $sample^{-1}$).
KEYWORDS: GTE, bioencapsulation, total polyphenol content, antioxidant activities, *Artemia* nauplii.

INTRODUCTION

Artemia nauplii are used extensively worldwide as live food for the larval stages of commercially important freshwater and marine fish species due to their availability, low cost, ease of culture and biochemical composition (Kolkovski *et al.,* 1997; Sorgeloos *et al.,* 1998; Smith *et al.,* 2004). The non-selective feeding behavior of *Artemia* also makes this organism a good biological carrier for transferring essential nutrients to predator larvae using bioencapsulation technique (Leager *et al.,* 1986; Citarasu *et al.,* 1998; Immanuel *et al.,* 2001, Immanuel *et al.,* 2004). The naupliar stages of *Artemia* are normally used in bioencapsulation because they can effectively filter feed small particulate up to 30 μm in size (Dobbleleir *et al.,* 1980; Smith *et al.,* 2004). During bioencapsulation, desired essential nutrients dissolved in water are ingested by nauplii with minimal amount of leakage when given to the fish or shrimp larvae. Thus, this technique is also developed not only for improving the quality of *Artemia* but for delivering water soluble antibacterial agents like oxytetracycline (Touraki *et al.,* 1995; Gomez-Gil *et al.,* 2001; Langdon *et al.,* 2003), oxolinic acid (Yahyazadeh *et al.,* 2007; Touraki and Niopas 2012) florfenicol (Roiha *et al.,* 2010) and metronidazole (Rodriguez *et al.,* 2011) to treat fishes affected by bacterial diseases in larval hatcheries. With emerging environmental risks of using artificial or synthetic drugs, interest in the utilization of plant extract as an alternative drug for aquatic animals is increasing. Plants contain phenolic compounds that have outstanding antioxidant and free radical scavenging properties (Hrelia *et al.,* 2002). Antioxidants have shown to have multiple functional and remedial properties that include anti-radical, anti-carcinogenic, anti-inflammatory oxidative stress reduction, and cardio protection (Chan, 2010). In addition to polyphenols, carotenoids present in plants were reported to serve as antioxidants and as source of vitamin A for animals (Ong and Tee, 1992; Miki, 1991; Briton, 1995; Ni *et al.,* 2008). Among the numerous plants exhibiting potential source of natural antioxidants, tea is an excellent source of polyphenol antioxidants (Hrelia *et al.,* 2002). Tea is a well-consumed beverage worldwide mainly because of the many beneficial health effects it has on human. Compared to black tea, green tea has been reported to have higher antioxidant capacity and a greater level of polyphenols (Koo and Cho, 2004). Aside from polyphenols, green tea also contains fibers, proteins, carbohydrates, fat, peptides, minerals and organic acid (Sato and Miyata, 2000; Bae and Lee, 2010). In aquaculture application, previous studies utilized green tea as immunostimulant by incorporating it in fish diet that can increase disease resistance, and improve survival rate, growth rate and antioxidant system (Sheikhzadeh, 2011). However, this method can only be used at the stage when the receiving animal starts feeding

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on formulated diets. For fish larvae, delivery of natural antioxidants from green tea can be done by first extracting them from the green tea leaves using water, then enrich the *Artemia* nauplii through bioencapsulation, and finally feed the nauplii to the larvae. The advantages of this method are its simplicity, lower economic cost and eco-friendliness. However, before giving it as a food it is important to know first if green tea extract was indeed enriched to *Artemia* nauplii during bioencapsulation. The aim of this paper was to determine the improvement of green tea extract to the total polyphenol content, total carotenoid and antioxidant capacity of *Artemia* nauplii.

MATERIALS AND METHODS

2.1. Sample preparation of green tea extract

A pack of commercial green tea leaves was purchased from a local supermarket in Tsukuba-shi, Japan. Tea sample was pulverized using coffee grinder and sieved at 120 μm. Green tea-water-infusion was prepared based on the method used by Komes *et al.,* (2010). Tea powder was mixed with distilled water (1mg/ml, w/v) at 80^{o}C and slowly stirred for 5 minutes. After extraction, the solution was cooled to room temperature and sieved at 120 μm. Salinity of tea extract was adjusted to 3.50% by adding artificial seawater powder and reserved for enrichment experiment.

2.2. Hatching of Artemia

Artemia salina cysts were obtained from a commercial supplier (A&A Marine) which originally came from Salt Lake, Utah USA. Hatching of cysts were carried out in 2g cysts/300mL of 3.50% artificial sea water (Benijts *et al.,* 1975). The 24h-incubation was conducted in thermo-controlled room set at 25^{o}C with light and vigorous aeration. Nauplii were separated from cyst shell for enrichment with green tea.

2.3. Bioencapsulation of Artemia nauplii with green tea water extract

Newly hatched *Artemia*, Instar I and Instar II naupliar stages (typically 0, 8 and 12 hours after hatching) were enriched in previously prepared green tea extract (GTE) at 3.50% salinity (Benjits *et al.,* 1975; Rodrigues *et al.,* 2011). Enrichment period was conducted for 6 hours, for it showed high results on a previous study by the team (not published), at thermo-controlled chamber set at 25^{o}C with light and vigorous aeration. Enriched *Artemia* nauplii were collected by sieving with 120-μm sieve, washed with tap water, then with distilled water, blot dried in paper towel and finally kept at -30^{o}C for further analysis.

2.4. Determination of total polyphenol content (TPC)

Extraction of TPC from *Artemia* nauplii was based on the method used by Anesini *et al.,* (2008) where about 0.2 g of each sample was weighed in an extraction tube, and 5 mL of 70% methanol at 70^{o}C was added. The extract was mixed using a vortex mixer and heated at 70^{o}C for 10 min. After cooling to room temperature, the extract was centrifuged at 200g for 10 min. The supernatant was decanted in a graduated tube. The extraction step was repeated and extracts were pooled and the volume was adjusted to 10 ml with cold 70% methanol. Estimation of TPC was done by colorimetric assay based on procedures described by Komes *et al.,* (2010). Sample (50 μL) was mixed with 2.5 mL Folin Ciocalteu's phenol reagent, 0.75 mL of 20% sodium carbonate and diluted to 5mL. After 2 hours, the absorbance of blue color solution was read at 725 *n*m using UV-VIS spectrophotometer (Ultrospec 3300 Pro, Amersham Biosciences). Amount of TPC was calculated using linear regression with gallic acid as standard reference (0-1000 mg L^{-1}). The results are expressed as mg gallic acid equivalents (GAE) per g of dry sample.

2.5. Determination total carotenoids

Extraction of pigment using acetone in nauplii was based on the method used by Buyukapar and Yanar, (2007), where about 10 mL acetone and 2g of anhydrous sodium sulfate were added to 1.0g of *Artemia* sample. The solution was mixed with a vortex mixer, and was then centrifuged at 5000 rpm for 5 minutes and stored for three (3) days at 4^{o}C in a refrigerator. After three (3) days of extraction, absorption of extract was measured at 470, 645 and 662 *n*m using UV-VIS spectrophotometer. The concentrations of C*a*, C*b* and total carotenoids were determined according to the equations reported by Lichtenthaler and Wellburn, (1983) as follows:

C*a* (mg L^{-1}) = 11.75 Abs$_{662}$ – 2.350 Abs$_{645}$

C*b* (mg L^{-1}) = 18.61 Abs$_{645}$ – 3.960 Abs$_{662}$

Total carotenoids (mg L^{-1}) = 1000 Abs$_{470}$ – 2.270 C*a* – 81.4 C*b*/227

2.6. Determination of antioxidant capacity

2.6.1. 1,1-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay

Scavenging activities of the extracts on the stable free radical DPPH were assayed based on the modified Blois method used by Bae and Lee, (2010) in which the bleaching rate of DPPH is

monitored at a characteristic wavelength in presence of the sample. A volume of 0.5ml of the sample extract was mixed with 4.5mL of 0.41mM DPPH solution in absolute ethanol. The mixture was kept for 30 minutes and then the absorbance was measured at 517 *n*m in UV-VIS spectrophotometer. A lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The DPPH scavenging activity was calculated using linear regression with L-ascorbic acid as standard reference (0-1000 mg L^{-1}). The results are expressed as mg vitamin C equivalents (VCE) per g of dry sample.

2.6.2. Total antioxidant (TA) activity

TA activity of the samples was analyzed according to the method used by Preito *et al.,* (1999) and Prasad *et al.,* (2009). In brief, a 0.1 mL-aliquot of the sample was mixed with 1 mL of the reagent solution (0.6 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and then incubated at 95°C for 90 min. After the samples were cooled to 25°C, the absorbance was measured at 695 *n*m against a blank. The blank contained 1 mL of the reagent solution without the sample. The total antioxidant activity was expressed as the absorbance of the sample. The higher absorbance value indicates the higher antioxidant activity. The total antioxidant activity was calculated using linear regression with L-ascorbic acid as standard reference (0-1000 mg L^{-1}). The results are expressed as mg vitamin C equivalents (VCE) per g of dry sample.

2.7. Statistical Analyses

Differences between treatments were performed using one-way analysis of variance ANOVA with Duncan's multiple range tests. The level of significance for all analyses was $P<0.05$. All treatments were conducted in triplicate unless specified. Results are all reported as dry matter and presented as mean ± standard error mean.

RESULTS

3.1. Total polyphenol content

Results of green tea bioencapsulation on TPC of *Artemia* nauplii using different naupliar stages are presented in Figure 1. TPC of *Artemia* enriched for 6hrs increases significantly ($P<0.05$) with the age of nauplii. Instar II nauplii contains the highest amount of TPC with 18.78 ± 0.45 and 20.08 ± 0.97 (mg GAE g^{-1} $sample^{-1}$) for control and GTE-enriched, respectively. However, no significant difference was observed between control and GTE-enriched nauplii at all stages.

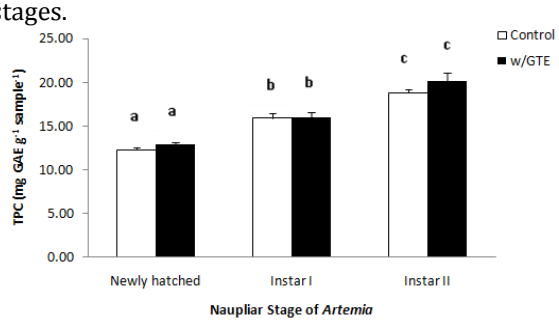

Figure 1: Total polyphenol content (TPC) of unenriched (control) and green tea extract (GTE) enriched *Artemia salina* nauplii at different naupliar stages. Each value is expressed as mean ± standard error mean (n=3). Values with different letter are significantly different ($P<0.05$) between the samples.

3.2. Total carotenoids

Total carotenoids of the acetone extract are summarized in Table 1. Results show that total carotenoid content of different naupliar stages of *Artemia* enriched for 6-hrs increases with age of nauplii for both control and GTE-enriched nauplii. Total carotenoids of Instar II nauplii for control (112.63 ± 2.00 mg g^{-1} $sample^{-1}$) and GTE-enriched (115.341 ± 4.86 mg g^{-1} $sample^{-1}$) are significantly ($P<0.05$) higher than newly hatched and Instar I nauplii. Although total carotenoids of GTE-enriched are higher than control no significant difference was observed. In this case, chlorophylls (*a* and *b*) from GTE may have a contribution to the total carotenoid values.

Table 1: C*a*, C*b* and total carotenoid of unenriched (control) and green tea extract (GTE) enriched *Artemia salina* nauplii at different naupliar stages

Naupliar Stages of *Artemia*	C*a* (x 10^{-2}) (mg g^{-1} $sample^{-1}$)		C*b* (x10^{-2}) (mg g^{-1} $sample^{-1}$)		Total Carotenoid (mg g^{-1} $sample^{-1}$)	
	Control	w/GTE	Control	w/GTE	Control	w/GTE
Newly Hatched	0.03 ± 0.03^{a}	0.53 ± 0.04^{b}	0.12 ± 0.10^{a}	0.38 ± 0.82^{ab}	83.46 ± 1.41^{a}	92.72 ± 2.21^{ab}
Instar I	0.18 ± 0.0.06^{a}	1.15 ± 0.02^{c}	0.38 ± 0.1.80^{ab}	0.44 ± 0.03^{ab}	92.43 ± 3.98^{ab}	99.79± 1.82^{b}
Instar II	0.07 ± 0.00^{a}	1.43± 0.08^{d}	0.16± 0.60^{a}	0.71 ± 1.79^{c}	112.62 ± 1.98^{c}	115.34 ± 4.86^{c}

*Each value is expressed as mean ± standard error mean (n=3).
**Values with different letter are significantly different ($P<0.05$) between the samples.

3.3. Antioxidant activities

In the present study, antioxidant activities of polyphenols extracted with 70% methanol were determined by DPPH free radical scavenging activity and TA activity. The antioxidant capacities of polyphenols are predicted

extensively using DPPH free radical scavenging activity because of the relatively short time required for analysis (Gulcin *et al.*, 2007). Figure 2 shows the DPPH free radical scavenging activity of *Artemia* nauplii and it could be noted that the nauplii enriched with GTE have significantly (P<0.05) higher value than their corresponding control. Highest values were observed for the GTE-enriched nauplii of Instar I and Instar II containing 71.64 ± 2.50 (mg VCE g^{-1} $sample^{-1}$) and 73.04 ± 1.95 (mg VCE g^{-1} $sample^{-1}$), respectively.

TA activity of *Artemia* nauplii at different naupliar stages are shown in Figure 3. As with the DPPH free radical scavenging activity, higher TA activity values could be observed for different naupliar stages enriched with GTE compared with their respective controls. Instar II of GTE-enriched nauplii contains highest TA activity amount of 18.08 ± 1.08 (mg VCE g^{-1} $sample^{-1}$), which is significantly (P<0.05) higher than the other samples.

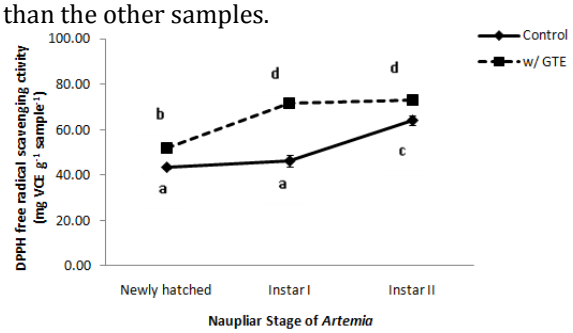

Figure 2: Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities of unenriched (control) and green tea extract (GTE) enriched *Artemia salina* nauplii at different naupliar stages. Each value is expressed as mean ± standard error mean (n=3). Values with different letter are significantly different (P<0.05) between the samples.

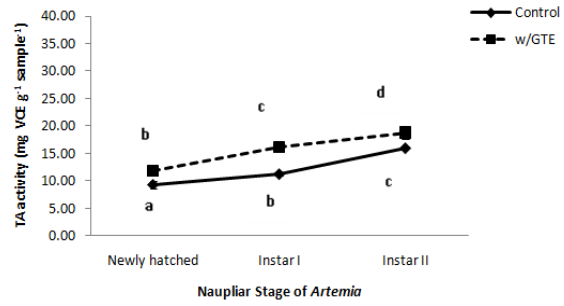

Figure 3: Total antioxidant (TA) activities of unenriched (control) and green tea extract (GTE) enriched *Artemia salina* nauplii at different naupliar stages. Each value is expressed as mean ± standard error mean (n=3). Values with different letter are significantly different (P<0.05) between the samples.

DISCUSSION

In the current study, bioencapsulation of green tea is not only done for the enhancement of antioxidant content of *Artemia* nauplii, but also, it is a way of preventing bacterial transfer carried by live food. The amount of 1mg/ml (w/v) of green tea powder was proven by Sornsanit, (2002) to treat *Vibrio*-infected shrimp culture water. *Vibrio* is known to cause mortality in larvae, post larvae, and juveniles of up to 100% of the affected population (Sunaryanto and Mariyam, 1987; Sarjito *et al.*, 2012).

Presently, there are no data in literature about the antioxidant activities of *Artemia* nauplii, i.e. on what causes it. In plants, on the other hand, it is known that the antioxidant activity of plant materials was due to the presence of phenolics, carotenoids and flavonoids (Barros *et al.*, 2007; Prasad *et al.*, 2009). Polyphenol compounds play an important role in adsorbing and neutralizing free radicals, quenching singlet oxygen, and decomposing peroxides by donating their hydrogen (Ksouri *et al.*, 2007). Without antioxidants like polyphenol, this group of radicals may interact with biological systems in a clearly cytotoxic manner (Barros *et al.*, 2007).

Bioencapsulation of green tea extract to *Artemia* nauplii, as presented in the present study, shows that the amount TPC encapsulated into/by the nauplii during the 6-hr enrichment proportionally increases with the age of nauplii. Higher results using Instar II nauplii can be attributed to the fact that at this stage, nauplii have already develop a functional digestive system and can obtain their sustenance and other extraneous substances by filter feeding on particles from the water (Makridis and Vadstein, 1999; Rodriguez *et al.*, 2011). This result agrees with the results obtained by Rodriguez *et al.*, (2011) where Instar II *Artemia* nauplii also accumulated the highest levels of metronidazole compared with the Instar I nauplii. Instar II nauplii were also used by Immanuel *et al.*, (2004) for *Odonus niger* lipid enrichment.

Generally, there was no significant difference observed between the TPC of the controls and that of the GTE-enriched samples, but their DPPH free radical scavenging activity and TA activity was statistically different. The improved DPPH free radical scavenging activity and TA activity on GTE-enriched samples can be attributed to polyphenols transferred from green tea to the *Artemia* nauplii during bioencapsulation. The carotenoids present in *Artemia* nauplii also contributes to the antioxidant activity. For the control *Artemia* nauplii, the antioxidant activity of the sample is probably due to carotenoids present in the sample.

In conclusion, enrichment of green tea in *Artemia* nauplii using bioencapsulation technique using different naupliar stages can improve its total polyphenol, total carotenoids and antioxidant activities. Best result was obtained when Instar II was used in the 6-hr enrichment. With the current results, it will be interesting to investigate the effect of these GTE-enriched *Artemia* nauplii when applied to fish or shrimp larvae to improve their antioxidant capacity against stressful conditions.

ACKNOWLEDGEMENT

The Monbukagakusho scholarship program of Japan for post-graduate studies is gratefully acknowledged.

REFERENCES

Anesini C, Ferraro GE, Filip R. Total polyphenol content and antioxidant capacity of commercially available tea (Camellia sinensis) in Argentina. J. Agric. Food Chem. 2008; 56: 9225–9229.

Bae MS and Lee SC. Effect of deep sea water on the antioxidant activity and catechins content of green tea. Journal of Medicinal Plants Research 2010; 4:1662-1667.

Barros L, Ferreira MJ, Queiros B, Ferreira I, Baptista P. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidants. Food Chemistry 2007; 103: 413-419.

Benjits F, Vanvoorden E, Sorgeloos P. Changes in the biochemical composition of the early larval stages of the brine shrimp, Artemia salina L. In: Persoone., Jaspers E. (Eds.), Proceedings- 10th European Symposium on Marine Biology Universa Press, Wetterean, Belgium 1975; 1-9.

Britton G. Structure and properties of carotenoids in relation to function. FASEB J. 1995; 9: 1551-1558.

Buyukacapar HM, Yanar M, Yanar Y. Pigmentation of rainbow trout (Oncorhynchus mykiss) with carotenoids from marigold flower (Tagetes erecta) and red pepper (Capsicum annum). Turk. J. Vet. Anim. Sci. 2007; 31:7-12.

Chan ES, Yim ZH, Phan SH, Mansa RF, Ravindra P. Encapsulation of herbal aqueous extract through absorption with ca-alginate hydrogel beads. Food and Bioproducts Processing 2010; 88: 3195–201.

Citarasu T, Immanuel G, Peter MM. Effect of feeding Artemia enriched with stressol and cod liver oil on growth and stress resistance in the Indian white shrimp Penaeu indicus postlarvae Asian Fish. Sci.1998; 12: 65–75.

Dobbleleir J, Adam N, Bossuyt E, Bruggeman E, Sorgeloos P. New aspects of the use of inert diets for high density culturing of brine shrimp. In: The Brine Shrimp Artemia 1980; 3:165-174.

Gomez-Gil B, Cabanillas–Ramos J, Paez–Brambila S, Roque A. Standardization of the bioencapsulation of enrofloxacin and oxytetracycline in Artemia franciscana Kellog, 1906. Aquaculture 2001; 196: 1–12.

Gulcin I, Elmastas M, Aboul-Enein HY. Determination of antioxidant and radical scavenging activity of basil (Ocimum basilicum L. Family Lamiaceae) assayed by different methodologies. Phytotheraphy Research 2007; 21: 354-361.

Hrelia S, Bardoni A, Angeloni, C, Leoncini E, Gallina Tochi T, Lercker G, Biagi, PL. Green tea extracts can counteract the modification of fatty acid composition induced by doxorubicin in cultured cardiomyocytes. Prostaglandins, Leukotrienes and Essential Fatty Acids 2002; 66: 519-524.

Immanuel G, Immanuel A, Palavesam M, Peter M. Effects of feeding lipid enriched Artemia nauplii on survival, growth, fatty acids and stress resistance of postlarvae Penaeus indicus Asian Fish. Sci. 2001; 14: 377–388.

Immanuel G, Palavesam A, Sivaram V, Babu M, Marian P. Feeding trashfish Odonus niger lipid enriched A.franciscana on growth, stress resistance and HUFA requirements of Penaeus monodon postlarvae. Aquaculture 2004; 237: 301-313.

Kolkovski S, Koven W, Tandler A. The mode of action of Artemia in enhancing utilization of microdiet by gilthead seabream Spanus aurata larvae. Aquaculture 1997; 155: 501-514.

Komes D, Horzic D, Belscak A, Ganic K, Vulic I. Green tea preparation and its influence on the content of bioactive compounds. Food Research International 2010; 43: 167-176.

Koo MW and Cho CH. Pharmacological effects of green tea on the gastrointestinal system. Eur J Pharmacol 2004; 500: 177-85.

Ksouri R, Megdiche W, Debez A, Falleh H, Grignon C, Abdelly C. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte Cakile maritime. Plant Physiology and Biochemistry 2007; 45: 244-249.

Langdon C. Microparticle types for delivering nutrients to marine fish larvae. Aquaculture 2003; 227: 259-275.

Leager DA, Bengston KL, Simpson P, Sorgeloos P. The use and nutritional value of Artemia as a

food source. Oceanogr. Mar. Biol. Ann. Rev. 1986; 24: 521–623.

Lichtenthaler HK,Wellburn AR. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions 1983; 11: 591–592.

Makridis P and Vadstein O. Food size selectivity of Artemia franciscana at three developmental stages. Journal of Plankton Research 1999; 21: 2191-2201.

Miki W. Biological functions and activities of animal carotenoids. Pure Appl. Chem. 1991; 63 :141–146.

Ni H, Chen QH, He GQ, Wu GB, Yang YF. Optimization of acidic extraction of astaxanthin from Phaffia rhodozyma. Journal of Zhejiang Univeristy Science B 2008; 9: 51-59.

Ong ASH and Tee ES. Natural sources of carotenoids from plants and soils. Meth. Enxymol. 1992; 213: 142-167.

Prasad KN, Yang B, Dong X, Jiang G, Zhang H, Xie H, Jiang Y. Flavanoid contents and antioxidant activities from Cinnamun species. Innovative Food Science and Emerging Technologies 2009; 10: 627-632.

Preito P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry 1999; 269: 337-341.

Rainha N, Lima E, Baptista J, Rodrigues C. Antioxidant properties, total phenolic, total carotenoid and chlorophyll content of anatomical parts of Hypericum foliosum. Journal of Medicinal Plants Research 2011; 5: 1930-1940.

Rodriguez L, Livengood EJ, Miles RD, Chapman FA. Uptake of metronidazole in Artemia at different developmental life stages. J. Aquat. Anim. Health 2011; 23:100-102.

Roiha IS, Otterlei E, Samuelsen OB. Evaluating bioencapsulation of florfenicol in rotifers (Brachionus plicatilis). Aquaculture Research 2010; 42: 1110–1116.

Sarjito, Ningrum NE, Radjasa OK, Prayitno SB. Application of repetitive sequence-based PCR on the richness of Vibrio on the tiger shrimp(Penaeus monodon Fab.). Journal of Coastal Development 2012; 15: 303-309.

Sato T and Miyata G. The nutraceutical benefit, Part I: Green tea. Nutrition 2000; 16: 315-317.

Sheilhzadeh N, Nofouzi K, Delaza A, Oushani AK. Immunodulatory effects of decaffeinated green tea (Camellia sinensis) on the immune system of rainbow trout (Oncorhynchus mykiss). Fish & Shellfish Immunology 2011; 31: 1268-1269.

Smith GG, Ritar AJ, Brown MR. Uptake and metabolism of a particulate form of ascorbic acid by Artemia nauplii and juveniles. Aquaculture Nutrition 2004; 10: 1-8.

Sorgeloos P, Coutteau P, Dhert P, Merchie G, Lavens P. Use of brine shrimp, Artemia spp., in larval crustacean nutrition: a review. Revs. Fish Sci.1998; 6: 55–68.

Sornsanit K, Nomura N, Migo VP, Matsumura M. The use of green tea powder and green tea polyphenols for treatment of Vibrio-infected shrimp culture water. J Jap Water Treat Biol 2002; 38: 111-115.

Sunaryanto A and Mariyam A. Occuraence of a pathogenic bacteria causing luminescene in penaeid larvae in Indonesia hatcheries. Bull. Brackish. Wat Aqua. 1987; 8: 64-70.

Touraki M and Niopass I. Bioencapsulation and pharmacokinetic study of oxolinic acid in rotifers (Brachionus plicatilis) Artemia franciscana nauplii and metanauplii Aquaculture Research 2012; 44: 701–712.

Touraki M, Rigas P, Kastritsis C. Liposome mediated delivery of water soluble antibiotics to the larvae of aquatic animals. Aquaculture 1995; 136:1-10.

Yahyazadeh M, Eimanfar A, Camargo WN, Soltani M, Mohebbi F. The use of oxolinic acid to enrich Artemia urniana from Urnia Lake, Iran. Research Journal of Biological Science 2007; 2: 221–224.