# COMPARATIVE CONTENTS OF MINERALS AND DIETARY FIBRES IN SEVERAL TROPICAL SEAWEEDS

Joko Santoso<sup>1)</sup>, Yumiko Yoshie-Stark<sup>2)</sup>, Takeshi Suzuki<sup>2)</sup>

#### Abstract

Nine species of Indonesian green, brown and red seaweeds were used in this experiment to study the distribution and contents of minerals and dietary fibres. The contents of macro and microminerals were detected by atomic absorption spectrophotometer and inductively coupled argon plasma emission spectrometer, respectively; whereas dietary fibres were determined according to modified enzymatic-gravimetric method. The contents of macromineral were dominated by Ca, K, Na and Mg, with values range were 11.95–28.31, 0.30–27.92, 0.66–25.74, 2.31–21.52 mg/g dry weight, respectively; whereas microminerals Cu, Fe and Zn were found in low concentration. The highest and smallest content of soluble dietary fibre were found in *Kappaphycus alvarezii* and *Halimeda macroloba*, respectively. *K. alvarezii* also had the highest content of total dietary fibre and percent soluble dietary fibre against total dietary fibre, whereas *Ulva reticulata* had highest content of insoluble dietary fibre.

Keywords: dietary fibre, Indonesia, mineral, seaweed

### INTRODUCTION

From the view point of foodstuff sources, seaweeds provide high nutritional compounds of minerals, fatty acids and free amino acids (Yoshie *et al.*, 1994; Yoshie *et al.*, 1995; Norziah and Ching, 2000), and also provide non-nutrient compound like dietary fibres (Suzuki *et al.*, 1993; Suzuki *et al.*, 1996, Wong and Cheung, 2000).

Seafood including seaweeds is known to be one of the richest sources of minerals. The most common minerals found in seafood are iodine, magnesium, calcium, phosphorus, iron, potassium, copper and fluoride (Ensminger *et al.*, 1995). Minerals are very important for the biochemical reaction in the body as a co-factor of enzyme. For examples, Ca, P and Mg build and maintain bones and teeth, whereas Na and K help maintain balance of water, acids and bases in fluids outside of cells, and involve in acid-base balance and transfer of nutrients in and out of individual cells, respectively (Ensminger *et al.*, 1995).

1)

Department of Aquatic Products Technology, Faculty of Fisheries and Marine Science, Bogor Agricultural University (IPB), Campus IPB Darmaga Bogor 16680 INDONESIA

Department of Food Science and Technology, Faculty of Marine Science, Tokyo University of Marine Science and Technology, Shinagawa Campus 4-5-7 Konan, Minato, Tokyo 108-8477, JAPAN

Defects in mineral nutrition are capable of producing severe impairment of health. For instances, Ca malnutrition causes abnormal bone formation, namely osteoporosis and anemia caused from Fe deficiency (Reinhold, 1988; Harel *et al.*, 1998; Martinez-Navarrete *et al.*, 2002). Deficiency in Mg can result in a variety of metabolic abnormalities, such as K depletion and clinical presentations (Schumann *et al.*, 1997).

Although dietary fibres belong to the non-nutritional compounds, it has been recognized as an important dietary constituent, which possesses a wide range of positive properties (Rim *et al.*, 1991; Leontowicz *et al.*, 2002). Dietary fibre has been reported to have several physiological effects, depending upon the individual sources (Dreher, 1987). High fibre diets are an important factor in the low prevalence of colon cancer, ischemic heart disease, diabetes mellitus, gallstones, hemorrhoids and hiatus hernia, and also improving large bowel function, increasing fecal bulk, etc (Eastwood, 1989; Schneeman, 1987). Different with dietary fibre from vegetables and fruits, dietary fibres in seaweeds contain some acidic group such as sulfuric group; therefore they have different characteristics in physicochemical and physiological effects, such as water holding capacity (Suzuki *et al.*, 1996; Wong and Cheung, 2000), oil holding capacity (Wong and Cheung, 2000), swelling capacity (Wong and Cheung, 2000), binding of vitamins and minerals (Yoshie *et al.*, 2000), binding of bile salts (Wang *et al.*, 2001), and lipid metabolism effect (Wang *et al.*, 2002).

Since Indonesia is located in tropical area; seaweed can grow up quickly, produce big biomass and no seasonal variation. However, they are considered an under-exploited resource, and are possible to use more. But, research activity about them is still rare. There is no available data about distribution and contents of minerals and dietary fibres. Therefore, the objective of the research is to study the distribution and contents of minerals and dietary fibres in some Indonesian green, brown and red seaweeds.

### MATERIALS AND METHODS

#### **Materials**

Nine species of Indonesian seaweeds were used in this experiment. Five green seaweeds (*Caulerpa racemosa*, *Caulerpa sertularoides*, *Cladophoropsis vauchaeriaeformis*, *Halimeda macroloba*, *Ulva reticulata*), three brown seaweeds (*Padina australis*, *Sargassum polycystum*, and *Turbinaria conoides*), and one red seaweed (*Kappaphycus alvarezii*) were collected from Seribu Islands, Jakarta Province.

# **Samples Preparation**

After removing sand, the seaweed samples were washed with clean seawater and transported to the laboratory under refrigeration condition. After washing with tap water and wiping with paper towel, seaweed samples were minced by a food cutter (MK-K75; Matsushita Electric Corp., Osaka, Japan), and stored at -20 °C until used.

# **Mineral Analysis**

To each sample was weighed 2 g (wet sample) in a Kjeldahl flask, added with 20 mL of concentrated nitric acid and the flask was left overnight. Five milliliters of concentrated perchloric acid and 0.5 mL of concentrated sulfuric acid were added, and then the flasks were heated until no white smoke remained. Samples were transferred into volumetric flasks using 2 % of hydrochloric acid, then analyzed by an atomic absorption spectrophotometer (Model AA-600, Shimadzu Co., Kyoto, Japan) with acetylene flame, single-slot head, and Pt-Rh corrosion resistant nebulizer for measuring the total contents of Mg, Ca, K and Na; whereas the contents of Cu, Zn, and Fe were measured using an inductively coupled argon plasma emission spectrometer (ICP Nippon Jarrel-Ash ICAP-757V, Kyoto, Japan).

All reagents were analytical grade and solutions were prepared using double-distilled deionized water. All glassware and plastic bottle were dipped in 2 % of Contaminon-L (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for at least 2 hrs, and rinsed with double-distilled deionized water to remove contaminants.

# **Dietary Fibres Analysis**

Soluble and insoluble dietary fibres were determined according to an enzymatic-gravimetric method (Prosky et al., 1988) which has been approved as the legal or recommended procedure for food analysis. However this method was modified here by using pancreatin (Suzuki et al., 1996; Plaami et al., 1989) because almost all seaweeds contain little protein and no starch. The procedure consists of following steps: (1) Boiling 2 g of wet sample with 30 mL of water for 5 min. (2) Incubation with 20 mL of 2 % pancreatin and 30 mL of phosphate buffer at pH 6.8 in the presence of NaCl (10 mM) for 24 h at 37 °C. (3) Water insoluble dietary fibre was filtered off by a glass fibre filter (GA-100, Adventec Toyo Inc., Tokyo, Japan), washed three times with 20 mL of 78 % ethanol, twice with 20 mL of 95 % ethanol and once with 10 mL of acetone, and dried at 105 °C. Water soluble dietary fibre was precipitated from the filtrate using 4 volumes of ethanol (at 60 °C) and recovered by filtration in the same way as for insoluble fibre. (5) All samples analyzed were assayed in duplicate and one of the duplicate was used to determine protein content by Kjeldahl method, while the other was used to determine ash content in the fibre precipitate. (6) The final corrected values or the amounts of dietary fibre were calculated by subtracting the weights of ash and protein from the dietary fibre precipitate.

# **Statistical Analysis**

Results are expressed as mean value  $\pm$  standard deviation. Comparison of means of three measurements, using a significant level of p < 0.05, was performed by analysis of variance and means separated by *F-test* and *Student's t- test* (Steell and Torrie, 1980).

### **RESULTS AND DISCUSSION**

### The Distribution and Content of Minerals

Table 1 shows the profiles and contents of macrominerals in some Indonesian seaweed samples. The high concentration of Na was found in *U. reticulata* (26.40 mg/g dry weight), *C. sertularoides* (25.74 mg/g dry weight), and *C. vauchaeriaeformis* (23.91 mg/g dry weight), whereas K as major mineral was found in *K. alvarezii* (87.10 mg/g dry weight), *T. conoides* (27.92 mg/g dry

weight), and *S. polycystum* (17.54 mg/g dry weight). The range contents of Ca and Mg were 2.80 to 28.31 mg/g dry weight and 2.38 to 21.52 mg/g dry weight, respectively. The highest and smallest concentrations of Ca were found in *P. australis* and *K. alvarezii*, whereas *U. reticulata* and *H. macroloba* contained the highest and smallest of Mg, respectively.

**Table 1.** The contents of macrominerals in seaweed samples (mg/g dry weight)

Seaweed Samples	Mg	Ca	K	Na
Green seaweeds				
Caulerpa racemosa	$3.84\pm0.32^{a}$	18.52±5.30°	$3.18\pm0.22^{b}$	$25.74\pm1.18^{d}$
Caulerpa sertularoides	$3.68\pm1.03^{a}$	11.95±4.43 <sup>b</sup>	$0.30\pm0.04^{a}$	$0.66\pm0.40^{a}$
Cladophoropsis vauchaeriaeformiss	$7.05\pm0.64^{b}$	22.28±3.28 <sup>d</sup>	$9.85\pm0.37^{c}$	23.91±1.24 <sup>d</sup>
Halimeda macroloba	$2.38\pm0.50^{a}$	$16.86\pm0.78^{c}$	$0.68\pm0.16^{a}$	$4.89\pm0.80^{b}$
Ulva reticulate	21.52±2.81°	$17.94\pm5.30^{c}$	$12.62\pm0.30^{c}$	$26.40\pm0.84^{d}$
<b>Bown seaweeds</b>				
Padina australis	$4.04\pm1.58^{ab}$	28.31±4.26 <sup>e</sup>	$0.53\pm0.19^{a}$	$0.97\pm0.85^{a}$
Sargassum polycystum	$5.69\pm0.66^{ab}$	18.67±1.44°	17.54±1.35 <sup>d</sup>	$9.65\pm1.36^{c}$
Turbinaria conoides	$5.74\pm0.26^{ab}$	14.79±2.17 <sup>b</sup>	27.92±1.08 <sup>e</sup>	11.47±0.45°
Red seaweed				
Kappaphycus alvarezii	2.88±0.34 <sup>a</sup>	2.80±0.31 <sup>a</sup>	87.10±5.83 <sup>f</sup>	11.93±2.47 <sup>c</sup>

Values are mean  $\pm$  standard deviation (n = 3)

Values within columns followed by different superscript letters are significantly different (p<0.05).

The profiles and contents of macrominerals in Indonesian seaweed samples were almost same to Japanese and Spanish seaweeds, and Na, Ca, Mg and K also became major minerals. In raw Japanese brown alga Laminaria japonica (ma-kombu in Japanese) the contents of Na, Ca, Mg and K were 590, 75, 120 and 42 mg/100 g edible portion (Resources Council, Science and Technology Agency, 1991). In another brown seaweeds (Fucus vesiculosus, Laminaria digitata, and Undaria *pinnatifida*) and red seaweeds (Chondrus cryspus and Porphyra tennera) grown in Spain, the concentration range of Na, Ca, Mg and K were 36.27-70.64, 3.90-10.05, 5.65-11.81, and 31.84-115.79 mg/g dry weight, respectively (Ruperez, 2002). Furthermore, the

content of Ca in *Gracillaria changii* grown in Malaysia was 651 mg/100 g wet weight (Norziah and Ching, 2000).

Table 2 shows the profiles and contents of micro minerals in some Indonesian seaweed samples. The concentration of Cu was from 0.002 mg/g dry weight (both in *S. polycystum* and *H. macroloba*) to 0.251 mg/g dry weight (in *C. sertularoides*). In other trace elements Fe and Zn, the content range were 0.032-0.813, and 0.003-0.227 mg/g dry weight, respectively. The highest content of Fe was found in *C. racemosa*, whereas *C. vauchaeriaeformis* contained the highest concentration of Zn.

**Table 2.** The contents of microminerals in seaweed samples (mg/g dry weight)

Seaweed Samples	Cu	Zn	Fe
Green seaweeds			
Caulerpa racemosa	$0.008\pm0.003$	$0.010\pm0.002$	0.813±0.237
Caulerpa sertularoides	$0.251 \pm 0.062$	$0.003 \pm 0.000$	$0.041\pm0.010$
Cladophoropsis vauchaeriaeformiss	0.227±0.003	0.227±0.003	0.111±0.056
Halimeda macroloba	$0.002 \pm 0.002$	$0.002\pm0.002$	$0.032 \pm 0.024$
Ulva reticulata	$0.179 \pm 0.001$	$0.017 \pm 0.001$	$0.280 \pm 0.060$
Brown seaweeds			
Padina australis	$0.005 \pm 0.001$	0.013±0.003	0.446±0.016
Sargassum polycystum	$0.002 \pm 0.001$	$0.004\pm0.001$	0.277±0.214
Turbinaria conoides	0.003±0.001	0.006±0.003	0.062±0.017
Red seaweed			
Kappaphycus alvarezii	0.005±0.000	$0.018\pm0.004$	0.070±0.033

Values are mean  $\pm$  standard deviation (n = 3)

## The Distribution and Contents of Dietary Fibres

Compared to Japanese seaweeds *Porphyra yezoensis* (susabi-nori in Japanese) and *Enteromorpha intestinalis* (ao-nori in Japanese), the contents of Fe and Zn were little bit higher than those in Indonesian seaweed samples, with range value 9.12-54.4 mg/100 g, 0.82-5.24 mg/100 g, respectively (Yoshie *et al.*, 1999). Furthermore, the contents of Fe and Zn in brown seaweeds (*F. vesiculosus*, *L. digitata*, and *U. pinnatifida*) and red seaweeds (*C. cryspus* and *P. tennera*) grown in Spain were almost same, and value range was 0.033-0.103; 0.017-0.071

mg/g dry weight, respectively (Ruperez, 2002), and in red alga *G. changii* contained Fe 95.6 mg/100 g wet weight (Norziah and Ching, 2000).

All these Japanese seaweeds also contained Cu in low concentration (0.46-1.82 mg/100 g edible portion = 0.006-0.023 mg/g dry weight) (Japan Society for Research of Food Composition, 1995). In Spanish brown and red seaweeds the content of Cu was also small, less than 0.005 mg/g dry weight (Ruperez, 2002).

The soluble, insoluble and total dietary fibre contents of nine Indonesian green, brown and red seaweeds are shown in Table 3. The soluble dietary fibre levels in K. alvarezii were higher than other seaweeds, and the value was 10.7 g/100 g dry weight, whereas C. vauchaeriaeformis and P. australis contained moderate amount, and the values were 4.2 and 4.9 g/100 g dry weight, respectively. C. sertularoides, S. polycystum and T. conoides were found to contain relatively small amount of soluble dietary fibre, and values were 1.8, 2.3 and 2.6 g/100 g dry weight, respectively. The lowest level of soluble dietary fibre was found in H. macroloba (0.4 g/ 100 g dry weight), followed by C. racemosa and *U. reticulata*, and each value was 0.9 g/100 g dry weight. On the other hands, K. alvarezii also contained the highest amount of total dietary fibre (69.3 g/100 g dry weight); however, the content of insoluble dietary fibre was not the highest in all of the samples. Green alga *U. reticulata* had the highest content of insoluble dietary fibre (64.8 g/100 g dry weight), followed by C. racemosa, S. polycystum, T. conoides, C. sertularoides and C. vauchaeriaeformis. The smallest content of insoluble dietary fibre was found in H. macroloba containing 14.3 g/100 g dry weight; this seaweed also had the smallest content of total dietary fibre, and the value was 14.7 g/100 g dry weight.

As for the percent soluble dietary fibre against total dietary fibre, *K. alvarezii* was the highest (15.4 %), followed by *C. vaucheriaeformis* (9.0 %) and *P. australis* (8.4 %). The moderate value was found in *H. macroloba*, *C. sertularoides*, *S. polycystum* and *T. conoides*, and the values were 2.5, 2.9, 3.5 and 4.2 %, respectively. The lowest values were found in *C. racemosa* (1.4 %) and *U. reticulata* (1.4 %).

**Table 3.** Dietary fibre contents of seaweed samples (mean  $\pm$  SD g/100 g dry weight)

Seaweed Samples	Total Dietary Fibre (TDF)	Insoluble Dietary Fibre (IDF)	Soluble Dietary Fibre (SDF)	SDF/TDF (%)
Green seaweeds				_
Caulerpa racemosa	$64.9 \pm 4.9^{de}$	$64.1\pm3.8^{e}$	$0.9\pm0.1^{ab}$	$1.4\pm0.1^{a}$
Caulerpa sertularoides	$61.8 \pm 1.1^{cd}$	$60.1\pm0.7^{de}$	$1.8\pm0.9^{ab}$	$2.9\pm1.5^{a}$
Cladophoropsis vauchaeriaeformis	47.0±4.8 <sup>b</sup>	42.8±4.8 <sup>b</sup>	4.2±1.1 <sup>cd</sup>	9.0±2.4 <sup>b</sup>
Halimeda macroloba	$14.7 \pm 1.6^{a}$	$14.3\pm1.8^{a}$	$0.4\pm0.3^{a}$	$2.5\pm2.1^{a}$
Ulva reticulata	$65.7 \pm 0.9^{de}$	$64.8 \pm 1.8^{e}$	$0.9\pm0.8^{ab}$	$1.4\pm1.2^{a}$
<b>Brown seaweeds</b>				
Padina australis	56.6±3.8°	$51.7 \pm 1.6^{c}$	$4.9\pm2.1^{d}$	$8.4\pm2.8^{b}$
Sargassum polycystum	$65.7 \pm 0.6^{de}$	$63.5 \pm 0.9^{de}$	$2.3\pm0.3^{abc}$	$3.5\pm0.6^{a}$
Turbinaria conoides	$63.7\pm3.6^{d}$	$61.0\pm4.5^{de}$	$2.6\pm1.0^{bc}$	$4.2\pm1.7^{a}$
Red seaweed				
Kappaphycus alvarezii	69.3±1.8 <sup>e</sup>	58.6±2.7 <sup>d</sup>	10.7±1.9 <sup>e</sup>	15.4±2.7°

Values are mean  $\pm$  standard deviation (n = 3)

Values within columns followed by different superscript letters are significantly different (p<0.05).

Compared to Japanese edible seaweeds, the soluble dietary fibre content in Japanese seaweeds was higher than that in Indonesian seaweeds, and range values were 7.2-25.6 g/100 g dry weight (Suzuki *et al.*, 1996), 7.1-25.1 g/100 g dry weight (Yoshie *et al.*, 2000) for Japanese seaweeds and 0.4-10.7 g/100 g dry weight for Indonesian seaweeds. On the contrary, Indonesian seaweed had the insoluble dietary fibre content (51.7-64.8 g/100 g dry weight) higher than Japanese seaweeds (15.6-58.6 g/100 g dry weight) (Suzuki *et al.*, 1996), (11.3-71.3 g/100 g dry weight) (Yoshie *et al.*, 2000), except *H. macroloba*. As a consequence, Japanese seaweeds also had higher value of soluble dietary fibre to total dietary fibre in percent than Indonesian seaweeds.

## **CONCLUSIONS**

From these results, it could be concluded that Indonesian seaweeds contained high number of macrominerals Na, K, Ca and Mg which were almost same to Japanese edible seaweeds, whereas the contents of microminerals Cu, Zn,

and Fe were low. Besides nutritional compounds (minerals), Indonesian seaweeds also contained non nutrient compounds *i.e.* dietary fibres.

Collecting or harvesting time may influence to the composition of nutrient and non-nutrient compounds, and in this experiment sample collection was carried out only in dry season (July); therefore the composition of nutrients and non-nutrients in Indonesian seaweeds has to be analyzed in different collecting or harvesting time (*i.e.* rainy season).

Since dietary fibres has positive properties for human health, therefore the research activities to prove whether its compounds from seaweeds also have same function in human, through animal or culture cells tested as a model are needed.

### **ACKNOWLEDGEMENTS**

The authors gratefully acknowledge the efforts of Mr. Satir for collecting seaweed samples, Dr. H. Oba from Laboratory of Aquaculture, Department of Aquatic Biosciences, Tokyo University of Marine Science and Technology for his help to identify seaweed samples.

#### REFERENCES

- Dreher ML. 1997. Handbook of Dietary Fibre. Marcel Dekker Inc., New York.
- Eastwood M. 1989. Dietary fibres in human nutrition. *J. Soc. Dairy Technol.* 42: 33-34.
- Ensminger AH, Ensminger ME, Konlande JE, Robson JRK. 1995. *The Concise Encyclopedia of Foods and Nutrition*. CRC Press, Boca Raton Florida.
- Harel Z, Riggs S, Vaz R, White L, Menzeis G. 1998. Adolescents and calcium: what they do and do not know and how much they consume. J. Adolescents Health. 22: 225-228.
- Japan Society for Research of Food Composition. 1985. Standard Tables of Dietary Fibres, Minerals, Cholesterols, and Fatty Acids in Japan. Ishiyaku Shuppan, Tokyo. Japan.
- Leontowicz M, Gorinstein S, Bartnikowska E, Leontowicz H, Kulasek G, Trakhtenberg S. 2001. Sugar beet pulp and apple pomace dietary fibres improve lipid metabolism in rats fed cholesterol. *Food Chem.* 72: 73-78.
- Martinez-Navarrete N, Camacho MM, Martinez-Lahuerta J, Mertinez-Monzo J, Fito P. 2002. Iron deficiency and iron fortified foods a review. *Food Res. Intern.* 35: 225-231.
- Norziah MH, Ching CY. 2000. Nutritional composition of edible seaweed *Gracilaria changgi. Food Chem.* 68: 69-76.

- Plaami S, Saastamoinen M, Kumpulainen J. 1989. Effect of variety of environment on dietary fibre content of winter rye in Finland. *J. Cereal Sci.* 10: 209-215.
- Prosky L, Asp NG, Schweizer TF, DeVries JW, Furda I. 1988. Determination of insoluble and total dietary fibre in foods and food product: Intercollaborative study. *J. Assoc. Off. Anal. Chem.* 71: 1017-1023.
- Reinhold JG. 1988. Problems in mineral nutrition: A global perspective. In: Smith KT (ed). *Trace minerals in foods*. Marcel Dekker Inc., New York. pp: 1-55.
- Resources Council, Science and Technology Agency. 1991. Standard Tables of Food Composition in Japan, Minerals (Magnesium, Zinc and Copper). Resources Council, Science and Technology Agency. Japan.
- Rimm EB, Ascherio A, Giovannucci E, Spiegalman D. Stampfer M, Willet W. 1996. Vegetables, fruits, and cereal fibre intake and risk of coronary heart disease among men. *J. Am. Med. Assoc.* 275: 447-451.
- Ruperez P. 2000. Mineral content of edible marine seaweeds. *Food Chem.* 79: 23-26.
- Schneeman, BO. 1987. Soluble vs insoluble fibre different physiological responses. *Food Technol.* 41: 81-82.
- Steell RGD, Torrie JH. 1980. *Principles and Procedure of Statistic. A Biometrical Approach*. McGraw-Hill Company, Inc. New York.
- Suzuki T, Nakai K, Yoshie T, Shirai T, Hirano T. 1993. Digestability of dietary fibre in brown algae, kombu by rats. *Nippon Suisan Gakkaishi*. 59: 879-884.
- Suzuki T, Ohsugi Y, Yoshie T, Shirai T, Hirano T. 1996. Dietary fibre content, water-holding capacity and binding capacity of seaweeds. *Fish. Sci.* 62: 454-461.
- Wang W, Onnagawa M, Yoshie Y, Suzuki T. 2001. Binding of bile salts to soluble and insoluble dietary fibres of seaweeds. *Fish. Sci.* 67: 1169-1173.
- Wang W, Yoshie Y, Suzuki T. 2002. Effect of small particle size on digestibility and lipid in rats. *Nippon Suisan Gakkaishi*. 68: 172-179.
- Wong KH, Cheung CK. 2000. Nutritional evaluation of some subtropical red and green seaweeds. Part I proximate composition, amino acid profiles and some physico-chemical properties. *Food Chem.* 71: 475-482.
- Yoshie Y, Suzuki T, Shirai T, Hirano T. 1994. Changes in the contents of dietary fibres, minerals, free amino acids and fatty acids during processing of dried nori. *Nippon Suisan Gakkaishi*. 60: 117-123.
- Yoshie Y, Suzuki T, Shirai T, Hirano T. 1995. Effect of sodium alginate on fat content and digestive organs of rats with fat free diet. *Fish. Sci.* 61: 668-671.