

Stability of Red Snapper (*Lutjanus Sp.*) Vitamin A Content as an Effect of Traditional Cooking Method of Asam Pedas

Meilisa Carlen Mainassy¹, Imanuel Berly Delvis Kapelle^{2*}

¹Departement of Biology, Faculty of Mathematic and Natural Sciences

²Departement of Chemistry, Faculty of Mathematic and Natural Sciences
Pattimura University, Ambon, Indonesia



Abstract - Red snapper (*Lutjanus sp.*) is significant for food-based strategy to alleviate vitamin A deficiency. Vitamin A content can be affected by cooking method. The study aimed to estimate vitamin A content of Red snapper which cooked by traditional cooking method of *asam pedas* (sour and hot). Scales, pectoral and ventral fins, gills, digestive tracts, and internal organs, except livers and gonads were discarded directly after fish were purchased from local market. Previously, fish were dipped in lemonade and salted water; then boiled in water with *asam pedas* spices. Fresh fish and *asam pedas* fish samples were extracted in two-replications, then were analyzed using high performance liquid chromatography (HPLC) at the wave length of 450 nm. Results shows that β -carotene content (average $\pm$ standard error) of fresh and *asam pedas* fish respectively was 3.30 ± 1.50 ; 0.41 ± 0.31 ; and retinol was 3.78 ± 0.14 ; 3.13 ± 0.01 $\mu\text{g/g}$. There were no significant differences ($p > 0.05$) between fresh and *asam pedas* fish of those two-carotenoids. Retinol activity equivalent (RAE) of fresh and *asam pedas* fish was 4.05 and 3.16 $\mu\text{g/g}$ wet weight respectively. The conclusion is *asam pedas* cooking method does not impair vitamin A content of Red snapper. It suggests that carotenoids of *asam pedas* spices contribute to sustain β -carotene and retinol contents during cooking.

Keywords- Red Snapper; β -carotene; Retinol; Vitamin A.

I. INTRODUCTION

Indonesia has very huge marine biological resources such as fish resources. Fish resources are for functional seafood based that has an economic value. One of reef fish which have an economic value as a source of carotenoids is Red Snapper (*Lutjanus sp.*). Red snapper belongs to Lutjanidae, the body is elongated and has a wide, convex head, and slightly concave. Red snapper commonly inhabits in reef waters into the estuaries tidal areas, and even some species can penetrate into freshwater [1].

Red snapper is consumed as source of protein. In addition to protein, this fish contains carotenoids that produce its red skin. These carotenoids compose of β -carotene and retinol that has biological function for health [2, 3]. Therefore, Red Snapper is a potential for food-based strategy to alleviate vitamin A deficiency. In some region of Indonesia, such as Moluccas Province, Red Snapper is served by *asam pedas* (sour and hot) traditional cooking.

The concentration of vitamin A can be affected by *asam pedas* cooking. Thus, the study aimed to estimate vitamin A content of Red Snapper which cooked by traditional cooking method of *asam pedas* and then to evaluate Red Snapper contribution to vitamin A requirement.

II. MATERIALS AND METHODS

A. Materials

Fresh fish was purchased from Fish Market, Semarang, and Central Java Indonesia. The *asam pedas* spices were obtained from local market, Salatiga, Central Java, Indonesia. The spices for *asam pedas* cooking are tomatoes (*Solanum lycopersicum*), chilli (*Capsicum annum*), liquid of Chinese lemon (*Citrus sp.*), garlic (*Allium sativum*), onion (*Allium ascalonicum*), leaves of basil (*Ocimum gratissimum*), root of ginger (*Zingiber officinale*), root of galangal (*Languas galanga*), and stem of lemon grass (*Cymbopogon nardus*). The chemicals used were acetone, hexane, methanol, KOH, ethanol, ethyl-acetate,

phenolphthalein, dicloromethan, acetonitrile, N₂ and distilled water.

B. Methods

1. Asam Pedas Cooking

Cooking method for *asam pedas* fish can be seen Figure 1. The quantities for each *asam pedas* spices as follows: 3 tomatoes, 4 Chinese lemons, 8 pieces of garlic, 4 pieces of onion, 10 basil leaves, 4 slices of ginger, 1 slices of galangal, 2 stems of lemon grass. Fish was cleaned by

removing scales, pectoral and ventral fins, gills, digestive tracts, and internal organs, except livers and gonads. Then, fish weight of 2 kg fishes was dipped in lemonade and salted water. For preparation, *asam pedas* spices were boiled in 2 L of water for 15 minutes; after that fish was boiled in *asam pedas* spices liquid for 20 minutes. Fresh fish and *asam pedas* fish samples were extracted in two replications then were analysed using high performance liquid chromatography (HPLC) at the wave length of 450 nm.

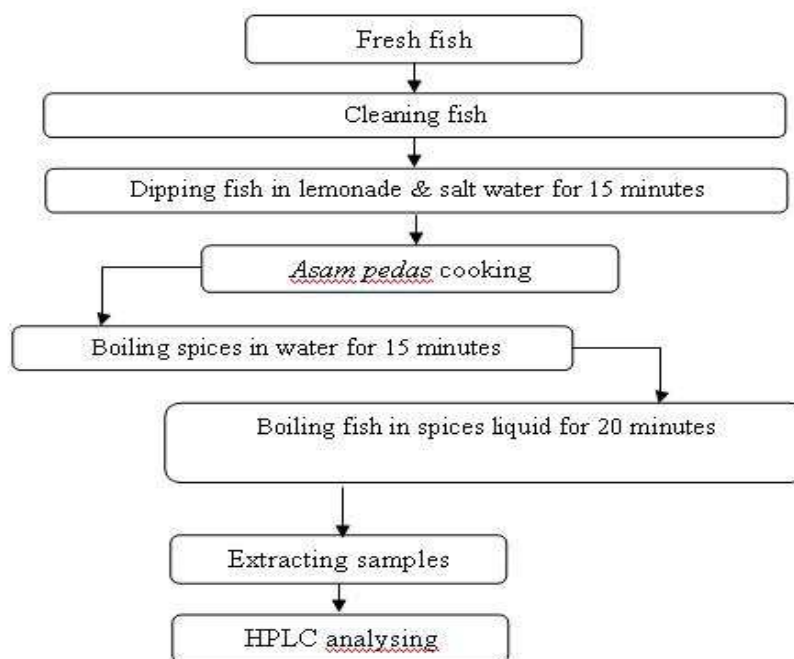


Figure 1. Flow Chart for *Asam Pedas* Cooking to HPLC Analysing

2. β -carotene Analysis

The β -carotene content of Red Snapper was analysed according to de Quirós *et al* and Raju *et al* [4, 5]. Around 5 g fish sample was added to 50 ml of acetone: ethyl acetate (2:1), then homogenized at low velocity for 10 minutes. Then, the sample was centrifuged for 5 minutes at 2500 rpm. The organic phase was evaporated in rotary evaporator of 250 ml boiling flask at 50°C. The dried sample was dissolved with acetone: ethyl acetate (2:1) at the level of 2 x 1 ml. Then, the solution was poured into 15 ml conical tube, dried under N₂, after that mixed with 1.0 ml methanol, filtered using Ø 0.45 µm Millex. Filtered sample solution of 20 µl was injected to HPLC. The β -carotene standard was done by adding 1.1 mg standard into 1 ml Eppendorf, then was dissolved with 1.0 ml acetone: ethyl acetate (2:1) to make 1100 ppm, later the standard solution was filtered

using Ø 0.45 µm Millex. The standard concentration was dissolved to 1.1 ppm to be injected to HPLC. HPLC was installed with Nova Pak C18, eluent was water: methanol = 50:50, flow was 1.2 mL/minute, detector was PDA, wave length was 450 nm, and temperature was 50°C.

3. Vitamin A Analysis

The vitamin A content of red snapper was analysed according to de Quirós & Costa (2006), Wall (2006), and Raju *et al.* (2007). Around 5 g was mixed with 25 ml alcohol KOH (10%), vortexes for approximately 1 minute, then reflux for 60 minutes. Subsequently, sample was extracted by means of n-Hexane in three stages of 50; 25; 25 mL. The sample was washed from KOH by water. Hexane phase was dried by evaporator rotary, and then was mixed with 3 ml n-Hexane. The sample was poured to 15 mL

conical to be dried again in water bath under N₂ flowing. Then, it mixed with 1.0 mL methanol, vortexes for 1 minute and centrifuged at 1500 rpm for 5 minutes. Then, it was filtered using Ø 0.45 µM Millex. Finally, 20 µL samples were injected into HPLC. For vitamin A standard, 0.0130 g vitamin A standard was put into 5 mL glass to make 2600 ppm standard solution. Then, 2.0 mL standard solution was mixed with 25 mL alcoholic KOH (10%) in 100 ml glass to be refluxed for 30 minutes. Subsequently, the standard solutions were extracted by means of n-Hexane in three stages of 50; 25; 25 mL. The solution was washed from KOH by water. The n-Hexane phase was dried by evaporator rotary, and then was mixed with 3 mL n-Hexane. The sample was poured into 15 mL conical to be dried again in water bath under N₂ flowing. Then, it mixed with 1.0 mL methanol to have standard solution of 5200 ppm. There were three standard solutions of 2.6, 5.2, and 10.4 ppm. These standard solutions were injected to HPLC at the volume of 20 µL. HPLC was installed with Nova Pak C18, eluent was water: methanol = 5: 59, flow was 1.2 mL/minute, detector was PDA, and wave length was 450 nm.

4. Retinol Activity Equivalent

Retinol Activity Equivalent (RAE) was calculated based only from β-carotene, it is 1 *retinol activity equivalent* = 12-µg β-carotene from food or 1-µg pure retinol. RAE is measurement unit for quantifying vitamin A contributed in diet by carotenoids [6].

C. Data Analysis

Experiment was done according to Complete Randomized Designed, with an *asam pedas* fish as treatment and a fresh fish as control. Each experiment unit extraction was duplicated. The data of β-carotene and retinol were analyses by t-test. Data were presented as average value.

III. RESULTS AND DISCUSSIONS

The β-carotene and retinol of fresh Red Snapper were not significantly different with those of *asam pedas* Red Snapper (Table 1). The results indicate that induction of high temperature, light, oxygen, and acid that occurred during *asam pedas* cooking did not significantly impair the stability of β-carotene and retinol. Those inductions are usually stimulating carotenoid degradation [7].

Table 1. The concentration β-carotene and Vitamin A of Red Snapper¹

No	Fish	β-karoten (µg/g ± SE)	Retinol (µg/g ± SE)
1	Fresh	3,30 ^a ±1,50	3,78 ^a ±0,14
2	<i>Asam pedas</i>	0,41 ^a ±0,31	3,13 ^a ±0,01

1. Averages with the same superscript were not significantly different using t-test (P>0.05)

There are two factors synergic sustain the stability of β-carotene and retinol. The first factor is the *asam pedas* spices such as chilli [8], Chinese lemon [9], tomatoes [10, 11] contain β-carotene that contribute to β-carotene of *asam pedas* fish.

The second factor is antioxidant of *asam pedas* spices protect β-carotene and retinol from degraded induction

during *asam pedas* cooking. Chilli contains phenol and volatile oil, basil contains phenol and catekin, ginger containd gingerol and shagaol [12], garlic contains allicin, caffeic acid, quercertin [13], lemon grass contains alpha-pinene, β-sitosterol, quercetin, saponin [13].

Table 2. Contribution of Red Snapper as functional food for vitamin A requirement

Life stage	Vitamin A requirement (µg RAE/individual/day)	Fish consumption (g/day)	
		Fresh	<i>Asam pedas</i>
Children 1–3 years	400	98.8	126.6
Children 4–5 years	450	111.1	142.4
Pregnant female	800	197.5	253.2
Lacting female	1,300	321.0	411.4

As functional food, Red Snapper can be used as source of vitamin A. Biological functions of vitamin A are for morphogenesis, vision, immunity, reproduction, neuron and neural development, maintenance of differentiation [2]. On averages, fresh and *asam pedas* Red Snapper provides respectively 4.05 and 3.16 µg RAE/g wet weight. The contribution of Red Snapper to the vitamin A requirement of people who are the most vulnerable to the vitamin A deficient [6] is shown on Table 2. This table reveals that the requirement of breast-feeding mother is 3.3 more than that of children of age 1–3 years. Therefore, the more is the vulnerability to vitamin A, the higher is the requirement for *asam pedas* Red Snapper.

IV. CONCLUSION

The method cooking *asam pedas* does not impair vitamin A content of Red snapper (*Lutjanus sp.*), which were analyzed using high performance liquid chromatography (HPLC) at the wave length of 450 nm. Results shows that β-carotene content (average ± standard error) of fresh and *asam pedas* fish respectively was 3.30 ± 1.50 ; 0.41 ± 0.31 ; and retinol was 3.78 ± 0.14 ; 3.13 ± 0.01 µg/g. There were no significant differences ($p > 0.05$) between fresh and *asam pedas* fish of those two-carotenoids. Retinol activity equivalent (RAE) of fresh and *asam pedas* fish was 4.05 and 3.16 µg/g wet weight respectively. It suggests that carotenoids of *asam pedas* spices contribute to sustain β-carotene and retinol contents during cooking.

REFERENCES

- [1] Saanin. 1984. *Taksonomi dan Kunci Identifikasi Ikan*. Bogor: Penerbit Bina Cipta.
- [2] De Luca, L. M. 1991. Retinoids and Their Receptors in Differentiation, Embryogenesis, and Neoplasia. *FASEB J* 5:2924–2933
- [3] Burri, B.J. 2011. Evaluating sweet potato as an intervention food to prevent vitamin A deficiency. *Comprehensive Reviews in Food Science and Food Safety* 10: 118–130.
- [4] de Quiro's, A.R-B. & H.S. Costa. 2006. Analysis of Carotenoids in Vegetable and Plasma Samples: A Review. *Journal of Food Composition and Analysis* 19: 97–111.
- [5] Raju, M., Varakumar, S. Lakshminarayana, R. Krishnakantha, T. P. & Baskaran, V. 2007. Carotenoid Composition and Vitamin A Activity of Medicinally Important Green Leafy Vegetables. *Food Chemistry* 101: 1598–1605.
- [6] USIOM (United States Institute of Medicine), Food and Nutrition Board. 2000. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington D.C.: National Academy Press.
- [7] Boon, C.S., D. J. Mc Clements, J. Weiss, & E.R. Decker. 2010. Factors Influencing the Chemical Stability of Carotenoids in Foods. *Critical Reviews in Food Science and Nutrition* 50:515–532.
- [8] Deli, J., Z. Matus, & G. To'th. 1996. Carotenoid Composition in the Fruits of *Capsicum annum* Cv. Szentesi Kosszarvú during Ripening. *Journal of Agriculture and Food Chemistry* 44: 711–716.
- [9] Yano, M. M. Kato, Y. Ikoma, A. Kawasaki, Y. Fukazawa, M. Sugiura, H. Matsumoto, Y. Oohara, A. Nagao, & K. Ogawa. 2005. Quantitation of Carotenoids in Raw and Processed Fruits in Japan. *Food Science and Technology Research* 11: 13–18.
- [10] Tee, E-S. & C-L Lim. 1991. Carotenoid Composition and Content of Malaysian Vegetables and Fruits by the AOAC and HPLC Methods. *Food Chemistry* 41: 309–339.
- [11] Aruna, G., B.S. Mamatha, V. Baskaran. 2009. Lutein Content of Selected Indian Vegetables and Vegetable Oils Determined by HPLC. *Journal of Food Composition and Analyses* 22: 632–636.
- [12] Shan, B., Y.Z. Cai, M. Sun, & H. Corke. 2005. Antioxidant Capacity of 26 spice Extracts and Characterization of Their Phenolic Constituents *Journal of Agriculture and Food Chemistry*. 53: 7749–7759.
- [13] Balch, P.H. & J.F. Balch. 2000. Prescription for Nutritional Healing, New York: Avery