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Nutritional Composition of Nutrient Density of Commonly-Consumed
Fish, Other Aquatic Animals, and Processed Fish Consumed by Women
and Children in Cambodia

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Table of Contents

1. Introduction	4
2. Materials and methods	4
2.1. Collection of Samples	4
2.2. Proximate Analyses of the Selected Samples	4
2.2.2. Estimation of Fat Content	4
2.2.3. Determination of Phosphorus	5
2.2.4. Determination of Iron	6
2.2.5. Determination of Protein	7
2.2.6. Determination of Moisture Content	7
2.2.7. Determination of Ash	7
2.2.8. Determination of Calcium	7
3. Results and Discussion	8
3.1. Protein Content	8
3.2. Fat Content	10
3.3. Ash Content	10
3.4. Moisture Content (MC)	10
3.5. Calcium Content	10
3.6. Phosphorus Content (%)	11
3.7. Iron Content	11
4. Conclusion	12
5. References	13

Abbreviation

OAA	Other Aquatic Animals
WHO	World Health Organization
FAO	Food and Agriculture Organization
ILCC	Industrial Laboratory Centre of Cambodia
AOAC	Association of Official Analytical Chemist
MC	Moisture content
P	Phosphorus content
Fe	Iron content
Ca	Calcium

1. Introduction

The food and nutritional consumption survey in women and children aged 5 years under activity plan within Investigation 5 “Enhancing food security and household nutrition vulnerability of women and children focus on nutrient dense commonly consumed fish from capture fish and aquaculture in Cambodia” under the project Titled: Improving Food Security, Household Nutrition, and Trade through Sustainable Aquaculture and Aquatic Resource Management in Cambodia and Vietnam. The survey founded that women commonly consume 43 of fish and OAAs species and preschooler commonly eats 38 of fish and OAAs species.

Thirteen commonly consumed fish species such as Trey Riel (*Cirrhinus caudimaculatus*), Trey Ros (*Chitala chitala*), Trey Kanhchos (*Mystus spp.*), Trey Chhpin (*Barbonymus gonionotus*), Trey Changwamoul (*Rasbora tornieri*), Trey Slat (*Notopterus notopterus*), Trey Andaing Toun (*Clarias microcephalus*), Trey Andaing Roueng (*Clarias batrachus*), Trey Chhlounh (*Macrognathus siamensis*), Trey Po (*Pangasius larnaudii*), Trey Chhlang (*Hemibagrus nemurus*), Trey Changvasrei (*Esomus longimanus*), and Trey Kranh (*Anabas testudineus*); three OAAs species namely fresh snail (*Pila ampullaceal*), fresh rice field shrimp (*Macrobrachium lanchsteri*), and fresh rice field crab (*Potamon sp.*); and two processed fish species smoked Trey Riel (*Cirrhinus caudimaculatus*), and sun-dried-salt Tre Ros (*Chitala chitala*);

The primary purpose of this activity is to determine the nutritional composition of nutrient density of the selected commonly-consumed fish species; OAAs; and processed fish which consumes by women and preschool children with focus key micronutrients (iron, calcium, and phosphorus) and macronutrient (Protein and Fat).

2. Materials and methods

2.1. Collection of Samples

Samples of fish was purchased or collected from public markets from Kompong Chhnang, Kandal, Takeo province and Phnom Penh city; and from Dai Fisheries in Tonle Sap River in Cambodia. The samples of fish were randomly selected and analyzed physiochemical properties in Industrial Laboratory Centre of Cambodia (ILCC), Ministry of industry in Cambodia.

2.2. Proximate Analyses of the Selected Samples

Fresh and processed samples were analyzed for their proximate composition using the standard analytical methods of Association of Official Analytical Chemist (AOAC 1990). Proximate analysis is a quantitative method of determining the macronutrients in food. This includes moisture, total ash content, crude fat, protein, calcium, iron and phosphorus content. The details on how proximate analyses were conducted in bellow:

2.2.2. Estimation of Fat Content

2.2.1.1. Reagents

- n-Hexane or Petroleum ether 40-60 fraction
- Pumice stone passing through mesh No 10 (Approx. 0.1 g per estimation)
- HCL :8M

2.2.1.2. Apparatus

- Mechanical mill to reduce particle size below 1 mm
- Balance of capacity 200 g and sensitivity 0.001 g

- Soxhlet extraction apparatus with flask size of 250 ml
- Cellulose thimbles 30 × 88 mm
- Oven operating at 103±2 °C
- Moisture estimation cans
- Desiccator with dehydrate silica gel

2.2.1.3. Sample preparation

- Estimation the moisture content of the sample
- Hydrolysis for release of bound fat
 - Boil the sample slowly with 45 ml distilled water in a beaker
 - Add 55 ml of 8M hydrochloric acid and few antibumping granules and stir
 - Boil gently for 15 min with a cover glass placed on the beaker
 - Rinse the cover glass with 100 ml water
 - Filter the content through a fluted filter paper
 - Continue to wash the residue with water till the filtrate is free of Chloride ions as shown by reaction with silver nitrate solution
 - Use the following table as guidance to select the amount of sample to be tested based on theoretical fat content of the food substabces.

2.2.1.4. Test procedure

- Weigh required amount 3-10g for testing directly into a thimble. In case of material subjected to pretreatment to hydrolyze the bound fast, transfer the filter paper and residue from sample preparation to a defatted extraction thimble
- Place thimble in a small beaker and dry at 105 °C for 6-8 hr
- Weigh a round bottom flask dried with antibumping granules at 105 °C
- Transfer the thimble with sample to a Soxhlet extractor (It may necessary to use a layer of defatted cotton wool on top of test material in the thimble to avoid floating of light particles).
- Add 180 ml hexane
- Reflux for 2-4 hr at rate of 3 cycles per hour (or 5-6 drops per second)
- Remove the flask and evaporate contents to dryness on a steam bath
- Dry the flask at 103±2 °C to a constant weight (usually 30-60 min) in an oven
- Allow to cool in desiccators and weigh

2.2.1.5. Calculation

$$\text{Fat\%} = \frac{\text{Mass of fat extracred}(g) \times 100}{\text{Dry weight}(g)\text{of test sample}}$$

2.2.3. Determination of Phosphorus

2.2.2.1. Prepare Reagent

- HCL:H₂O(1+3)=HCl 120ml and water 60ml and mix together
- Molybdovannadate reagent : Solution(NH₄)₆MO₇O₂₄: 2g/20ml in hot water +NH₄VO₃:0.1 g/12.5ml in hot water+ 1ml HCL(1:3) dilute to 100ml by distill water
- Stock Standard Solution : KH₂PO₄: Dry 2h at 105 °C, weigh = 0.8587 g/100ml in H₂O : 2 mg/ml
- Working Standard Solution : KH₂PO₄ :0.1 mgP/ml

2.2.2.2. Prepare Sample

Weigh sample 1g put in Crucible to dry on hot plate, then transfer to furnace for 3 hr then keep cool and add 4 ml HCL (1:3) and transfer to volumetric flash 100 ml and dilute to 100 ml

- Sample: pipette solution a above 1 ml put in volumetric flash 20 ml , and add 2 ml Molydobvannadate reagents dilute to 20 ml keep 1 hour and Measurement at wave length 400 nm
- Bland: Pipette solution an above 1 ml put in volumetric flash 20 ml add dilute to 20 ml keep1 hour and Measurement at wave length 400 nm.

2.2.2.3. Calculation

$$P\% = \frac{X(sam - BI) \times 1000ml \times 100}{Weigh(g) \times 1 \times 1000}$$

2.2.4. Determination of Iron

2.2.3.1. Reagent

Standard Fe solution:

- Iron II solution (20 ug Fe/ml) (Dissolve 0.014 g Ammonium Iron II sulfate hexahydrate in 2 ml water and 2 ml H₂SO₄ (9 mol/L:498.6 ml/L) and make up to mark 100 ml)
- Ortho Phenantroline solution : (2.5 g/l): Dissolve 250 mg of O-phenantroline in 100 ml of water
- Hdroxylammonium chloride solution : (5 g/10ml): Dissolve 5 g Hydroxylammonium chloride (HONH₃CL) in 10 ml of water
- Sodium acetate solution: (4 mol/l): Dissolve 27.5 g CH₃COONa.3H₂O in 50 ml of water.
- Sulfuric acid: (1 mol/l: 55.4ml/l): Dissolve 98 g H₂O in 1 L of water

2.2.3.2. Procedure

- Sample preparation

Pipette 0.14 ml of sample into 20 volumetric flask and add 0.2 ml of C, 2 ml of D, 1 ml of B and dilute to 20 ml by distil water.

- Blank sample

Pipette 0.14 ml of sample into 20 volumetric flask and add 0.2 ml of C, 2 ml of D, and dilute to 20 ml by distil water.

2.2.3.3. Determination

Measure Fe content in food by using spectrophotometer with absorbance at 510 nm 1 cm cell of water, before measure keep about q hour.

2.2.3.4. Calculation

$$Fe(ppm) = X(sam - BI) \times 25ml / (1ml \times 0.5g)$$

2.2.5. Determination of Protein

Weigh the sample 70-90 mg into the Kjeldahl flask. Add 3.6 g potassium sulphate, 0.2 g copper sulphate, 2.7 ml sulphuric acid and boil 400 °C for 2 hr. Allow to cool and dilute with 40 ml distilled water. Add 15 ml of hydroxide solution 40 %. Then drop Methyl blue mixed with Methyl red 3 drop and 5ml of boric acid. Distill it on distiller. Titrate with Hydrochloric acid 0.02 M until it change color from blue to pink and notice then calculate it by using formula:

$$\% \text{Protein} = \% \text{N} \times 6.25$$

2.2.6. Determination of Moisture Content

Heat the aluminum can in the oven at 105 °C for 1h and store in the desiccator for 15 mn. Weigh the sample 2 g into the aluminum can. Then transfer into the oven 105 °C for 3h. Keep the sample into the desiccator for 15 mn. Weigh the dry sample.

Calculate by:

$$\% \text{Moisture} = \frac{M_1 - M_2}{M_1} \times 100$$

2.2.7. Determination of Ash

Heat the crucibles in the oven at 105 °C for 1h and store in the desiccator for 15 mn. Weigh the sample 2 g into the crucible. Then transfer into the furnace oven 605 °C for 2 hr. Transfer the sample with crucible into the desiccator for 15 mn. Weigh the dry sample.

Calculate by:

$$\% \text{Ash} = \frac{M_2}{M_1} \times 100$$

2.2.8. Determination of Calcium

Weigh the sample 2 g into Crucible and transfer to furnace oven at 600 °C 1h, Add H₂O₂ 2 ml and transfer again into furnace oven 1h, add HCL 5 ml. Then transfer to volumetric flask 50 ml and dilute to 50 ml. Pipette 10 ml in to Erlenmeyer flask. Add NaOH 5 ml, indicator Murexide 5 % shake it and titrate with EDTA 0.05 N.

Calculate by:

$$Ca = \frac{V_{EDTA} \times C_{EDTA} \times 20 \times 1000 \times F_{EDTA} \times 50}{M_{sample} \times V_{sample}}$$

3. Results and Discussion

Table 1. Summary of nutrient density of macronutrients and micronutrients in fresh and processed fish species

Nutrients	Fresh		Processed	
Macronutrients	Min (%)	Max (%)	Min (%)	Max (%)
Protein	10.11	16.81	37.38	38.52
Fat	0.99	4.25	6.01	24.65
Micronutrients	Min (mg/100g)	Max (mg/100g)	Min (mg/100g)	Max (mg/100g)
Calcium	15	123.9	19.1	83.3
Phosphorous	0.57	3.98	1.15	2.06
Iron	0.26	0.83	0.3	0.38

Table 1 shows the nutrient density value of thirteen fresh water fish species, 3 OAAs (rice-field snail, rice-field shrimp and rice-field crab); and two processed fish (smoked *Henicorhynchus sp.*, and sun-dried-salt *Channa sp.*).

Fresh and processed fishes proximate analysis was administered for content of protein, fat, ash, moisture content, calcium, Iron and phosphorus. The results were as follows:

3.1. Protein Content

Fish are an excellent source of protein, a macronutrient that provides all the essential amino acids that our body needs. Proteins are made up of amino acids that are the body's building blocks. These amino acids tend to break down and need to be replaced on a daily basis by eating foods that are rich in protein.

Protein can be found in both animal and plant foods. However, the concentration of protein is higher in animal foods. For example, 100 g of raw fish has 14-20 g of protein depending on the species compared with only 2.7 g in cooked rice or 8.7 g in cooked beans.

Fish and meat are also more efficient sources of protein. This means that your body is better able to absorb the protein contained in these animal foods compared with plant foods. For many households, fish is more accessible and affordable than meat. People can raise fish in small ponds and rice fields or catch wild fish even if they do not have money to buy fish.

The protein content in fresh and processed fish found that the protein content was varied from 10.11 % to 16.81 % for fresh fish and 37.38 % to 38.52 % for processed fish (Table 2). Although, the protein content was increased after processed fish. It can be concluded that the protein content was not lost during drying and smoke. The slight increase in protein contents after drying may be due to product dehydration which concentrated proteins, thus increasing the nutritional value of fish. According to Ahmed *et al.* (2011), fresh fish had lower amounts of protein between 18.81 and 21.23 g/100 g as compared to smoked-dried fish between 69.10 and 75.72 g/100g.

Table 2. Nutritional composition of nutrient density of the selected commonly-consumed fresh and processed fish species eaten by women and preschooler

Loca/Common Name	Scientific Name	Protein (%)	Fat (%)	Ash (%)	MC (%)	Ca (mg/100g)	P (%)	Fe (mg/100g)
Fish Species								
Trey kanhchos	<i>Mystus spp.</i>	13.09	3.70	3.48	76.38	66.1	3.60	0.33
Trey Chhpin	<i>Barbonymus gonionotus</i>	16.05	3.46	3.69	67.43	46.9	0.57	0.34
Trey Changwamoul	<i>Rasbora tornieri</i>	16.40	1.74	3.48	68.29	62.1	3.89	0.35
Trey Riel	<i>Cirrhinus caudimaculatus</i>	13.22	4.25	3.16	70.95	58.2	1.05	0.21
Trey Ros	<i>Chitala chitala</i>	15.36	1.61	4.26	76.40	15.0	1.15	0.27
Trey Slat	<i>Notopterus notopterus</i>	13.64	1.00	25.66	74.34	102.4	3.97	0.26
Trey Andaing Toun	<i>Clarias macrocephalus</i>	14.81	1.00	2.45	73.62	45.3	3.01	0.26
Trey Andaing Roueng	<i>Clarias batrachus</i>	14.22	1.00	2.20	63.42	87.3	3.06	0.28
Trey Chhlounh	<i>Macrognathus siammensis</i>	14.07	1.00	25.33	74.67	91.1	3.85	0.30
Trey Po	<i>Pangasius larnaudii</i>	10.11	1.00	3.54	68.34	72.5	3.20	0.24
Trey Chhlang	<i>Hemibagrus nemurus</i>	15.30	0.99	2.17	73.82	123.9	3.48	0.30
Trey Changvasrei	<i>Esomus longimanus</i>	16.81	1.00	2.21	73.51	97.9	3.98	0.35
Trey Kranh	<i>Anabas testudineus</i>	15.99	1.00	3.37	69.51	82.3	3.70	0.27
Other Aquatic Animals								
Fresh Rice-Field Snail	<i>Pila ampullacea</i>	11.63	1.44	2.4	84.48	66.1	1.04	0.83
Fresh Rice-Field Shrimp	<i>Macrobrachium lanchsteri</i>	12.01	1.64	2.92	82.16	60.5	0.65	0.34
Fresh Rice-Field Crab	<i>Potamon sp.</i>	13.82	2.65	13.34	67.03	56.0	0.75	0.50
Processed Fish								
Smoked Trey Riel	<i>Cirrhinus caudimaculatus</i>	38.52	24.65	10.20	20.86	83.3	2.06	0.38
Sun-dried-salt Trey Ros	<i>Chitala chitala</i>	37.38	6.01	22.78	27.59	19.1	1.15	0.30

3.2. Fat Content

Results of fat analysis showed that the fat content ranged from 0.99 % to 4.25 % in fresh fish and 6.01 % to 24.65 % in processed fish (Table 2). Fresh fish flesh appeared to be relatively low in fats and this is also due to the concentration of other components of fish after drying. According to Ahmed *et al.* (2011) fat content are slightly raised after sun-drying and smoke-drying.

3.3. Ash Content

There were highly observed in ash content of crab (13.34 %), Treyros dried fish (22.78 %), Trey Chhlounh fish (25.33 %), and Trey Slat fish (25.66 %). Fresh fish had low in ash content (2.17 % to 4.26 %), Ahmed *et al.* (2011) found out that ash content of smoked-dried fish were high, 8.13-9.86 g/100 g, while much lower values were obtained for fresh fish. Probably the high ash values of sun-dried or smoke-dried fish flesh because of water loss during drying that concentrated other components of fish like its mineral contents (Table 2).

3.4. Moisture Content (MC)

The moisture content of Trey Andeng Reung ranged from 63.42 % to 84.48 % in fresh fish and from 20.86 % to 27.59 % in processed fish (Table 2).

The moisture content of the fresh fish is comparable to the studies conducted by Ahmed *et al.* (2011), however dried samples (around 79-80 % MC) in this study contained considerably high values. Ahmed *et al.* (2011) reported that moisture content varied between 81.49 and 84.33 g/100 g for fresh fish; between 7.58 and 8.95 g/100 g for smoked-dried fish and between 11.5 and 14.06 g/100 g for sun-dried fish. This could be explained by the fact that during smoke-drying the flesh loses water in the initial phase. On the contrary to sun-dried fish which tends to absorb moisture from high ambient air humidity, the protective coating of smoked-dried fish minimize rehydration.

The effect of traditional drying processes on the nutritional values of fish was studied by Eves and Brown (1993). It has been observed that different processing and drying methods have different effects on nutritional compositions of fish. This is because heating, freezing and exposure to high concentration of salt lead to chemical and physical changes and therefore digestibility is increased, due to protein denaturation, but the content of thermolabile compounds and polyunsaturated fatty acids is often reduced (George, 2012).

3.5. Calcium Content

Fish bones are very rich in calcium which your body needs to develop and maintain strong bones and teeth. The popular Cambodian meal of small fish species that are fried and eaten whole including the head and bones is an important dietary source of calcium. Teenage boys and girls need calcium because they are growing rapidly at this age. A serving of 100 g of small fish species such as *chanwa phlieng* or *chanwa mool* will provide 50 % of their daily requirement. The same serving will provide 70% of the daily requirement for adult men and women.

The calcium content in fresh fish ranged from 15.00 to 123.9 mg/ 100 g (Table 2). It was similar to Kotchanipha *et al.* (2012) reported that fresh fish had calcium 111.92 mg/100 g. On the other hand, processed fish was contained between 19.1 to 83.1 mg/100g which it was link with Kotchanipha *et al.* (2012) as processed fish 60 mg/100 g. According to ASEAN Food Consumption Table (2000) reported that calcium contained 44 mg/100 g for fresh fish and 274 mg/100 g for processed fish. However, Dipak *et al.* (2013) reported that calcium content was 52.79 mg/100 g in fresh fish.

Small fish are eaten whole, including the bones, and are, therefore, a rich calcium source. Large fish do not contribute to calcium intake because their bones are discarded as plate waste and not eaten (Roos, 2001).

In Cambodia, it was recorded that the majority (80 %) of the households cooked the commonly consumed fish, *Trey Changwa Plieng*, with the head intact. The contents of calcium and iron were considerably higher (58, and 25 %, respectively) in raw, cleaned samples, with the head than in samples in which the head was discarded during cleaning (Thorseng and Gondolf, 2005). The calcium in small fish has been shown to have the same high bioavailability as that from milk in both humans and rats (Hansen *et al.*, 1998; Larsen *et al.*, 2000).

3.6. Phosphorus Content (%)

The phosphorus content was found ranging from 0.57 to 3.89 mg/100g in fresh fish and 1.15 to 2.06 in processed fish (Table 2). According to Kotchanipha *et al.* (2012) reported that fresh fish had calcium 50.54 mg/100 g as compared to processed fish 23.48 mg/100 g. However, phosphorus content contained 0.15 % for fresh fish and 0.53 % for processed fish (ASEAN Consumption Table, 2000).

3.7. Iron Content

The iron content ranged from 0.21 to 0.83 gm/100 g in fresh fish and 0.30 to 0.38 mg/100g in processed fish (Table 2). According to ASEAN Food Consumption Table (2000) reported that iron content contained 0.12 mg/100g in fresh fish and 0.2 mg/100g in processed fish. Iron in fish is present in the forms of heme iron, a high-molecular subpool of complexbound nonheme iron, and inorganic iron, the proportions varying with fish species (Roos *et al.*, 2007).

Analysis of 16 common Cambodian fish species showed that, on average, 30 % of the iron in these fish was present as inorganic iron, the remainder being heme iron and complexbound nonheme iron. The bioavailability of heme iron is estimated as 25 %, 25 % for complex-bound nonheme iron, and 10% for inorganic iron (Roos *et al.*, 2007).

4. Conclusion

- Protein content varied from 10.11 % to 16.81 % in fresh fish and from 37.38 % to 38.52 % in processed fish.
- Fat content ranged from 0.99 % to 4.25 % in fresh fish and from 6.01 % to 24.65 % in processed fish.
- Calcium content ranged from 15.0 to 123.9 mg/100 g in fresh fish and from 19.1 to 83.3 mg/100 g in processed fish.
- Phosphorus content ranged from 0.57 to 3.89 mg/100g in fresh fish and from 1.15 to 2.06 in processed fish.
- Iron content ranged from 0.21 to 0.83 gm/100g in fresh fish and from 0.30 to 0.38 mg/100g in processed fish.

In conclusion, fish are high nutrient contents of protein, calcium, iron and phosphorus and it is safe to consume which are commonly consumed by the poor in rural area in Cambodia.

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