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Research Article

Proximate Composition Analysis of Some Commercial Dried Fishes Sold in Malaysian Markets

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Abstract

The prime reason for fish drying is to reduce the moisture content of the fish to avoid the growth of microorganism. According to the terrain of the area and the monsoon climate, the amount of fish caught throughout the year was usually not stable to consume. Thus, some of the fresh fish is preserved become dried fish products to prolong the storage period of fishes. The aim of the study was to compare the biochemical constituents and microbial load for the selected dried fishes (*Rastrelliger kanagurta, Megalaspis cordyla* and *Otolithes ruber*) that are commonly consumed by the Malaysian people which was bought in 2 different places in Kuala Lumpur Malaysia markets. All 3 dried fish samples for the present study was bought from 2 places were homogenized with 0.1 M phosphate buffer and used for the biochemical analysis. About 2 g of weighed sample was taken directly for ash and moisture estimation. Fibre was detected by using tri-chloro acetic acid method and salt content was measured using Silver Nitrate and Potassium thio-cyanate solution. Carbohydrate was estimated by DNS method. Estimation of protein was done by Lowry method and the fat was estimated by Soxhlet method. Calcium was measured by titrimetric method while phosphorus was estimated by mixing the sample with ammonium molybdate and Stannous Chloride method. From the nutritional value in dried fish, the fibre content having the highest value then followed by moisture and salt content while phosphorus content showed the lowest value. From the comparison between the 3 types of dried fishes, *Megalaspis cordyla* having the highest value in 7 among 9 parameters which were ash, moisture, salt, protein, carbohydrates, calcium and phosphorus test.

Keywords: Dried fish product, nutritional value, biochemical tests, minerals, carbohydrates, microbial load.

Introduction

The beneficial effects on health by including fish in diet are well known and have been documented in several studies. Throughout the world, it is well accepted that fishes are good sources of animal protein and other elements for the maintenance of health and body. Besides protein sources, small indigenous species of fishes are also a rich source of minerals such as calcium and phosphorus (Amin *et al*., 2013). *Rastrelliger kanagurta*, also known as Indian mackerel; widely distributed around the world and has good domestic demand as one of the food fish. Among variety types of fishes in Malaysia market, *Rastrelliger kanagurta* is one of the important fish. It is locally named as 'Kembung' and can be made in many forms of product like fresh, dried, canned, salted, and smoked on the coast of Malaysia. This type of fish is very popular due to the low market price, high abundance and easily found in market (Amin *et al.,* 2013).

Megalaspis cordyla, also known as hardtail scad, is one of the major fishes throughtout the markets in Indo-Pacific, including Malaysia. On the basis of statistics, the biggest utilizer of this species is Indonesia, with a take of 42,000 tonnes followed by Malaysia with a take of 22, 000 tonnes. *Megalaspis cordyla* is a predatory fish which both consumes larger prey like cuttlefish and squid and filter feeds to consume planktonic organism. It is one of the major important fish and usually marketed fresh, dried or salted (Simon *et al*., 2014). *Otolithes ruber*, also known as tiger tooth croaker, is neritic fish and lives in coastal waters over muddy bottoms (Sahar *et al*., 2014). Top of pectoral fin base before the origin of pelvic fins base and below the tip of gill cover which origin of pelvic fin before dorsal fin origin. These fish eat tiny crustaceans, fish, shrimp and sea bottom organism (Eskandari *et al*., 2012).

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Fish drying is one of the processes in fish meal production. For countries like Thailand, Vietnam and Malaysia which located adjacent to the largest freshwater in the region, fresh fish is a source of significant nutrients for the residences. According to the terrain of the area and the monsoon climate, the amount of fish caught throughout the year was not stable for consuming, so wisdom of fresh fish preservation for protein source had arisen when the amount of fresh fish was in a high production in a season.
Normally, salting and drying are used as method which also is traditional way to preserve fish product. Drying is a method of food preservation which involved indoor drying and outdoor drying. It mainly works by removing water from the food by using evaporation like smoking, sun drying and wind drying. Bacteria, yeasts and moulds need the water in the food to grow, and drying effectively prevents them for surviving in food (Abbas *et al.*, 2009). But this method causes the possibility of contamination and unhygienic in direct sunlight. So, combination of drying with salt, fish can be preserved and creating an environment inhospitable for bacteria when salt content increasing. This is because salt can kills bacteria at the cellular level by dehydrating them. Other than that, due to the large amount of dried fish product involved in different types of food, thus methods of packing the dried fish also very important. The packing material is specially made for storing dried fish so that dried fish can keep in protection from the bacteria, fungus and other microorganism. Fish product is low cost dietary protein sources and used as a substitute of fish at the scarcity of fresh fish. It is believed that the nutritional value and the physical properties of dry fish will be deteriorated with the increasing of storage period. The main reason for fish drying is to reduce the moisture content of the non-aqueous material. This is due to the dry fish absorb moisture rapidly in monsoon period and become suitable for infestation by beetles and mites. But, fish drying process affect the protein quality very easily (Abolagba and Melle, 2008). This is because nutritional value is affected when exposed to high temperature for longer period. If the storage temperature is kept below 70°C during the process, the drying time and heat have an impact on the protein quality (Tee *et al.*, 1987).

Numerous researches have shown that fish is good for the health. Based on health results for the last 50 years, a group of American scientist have concluded that consumption of fish minimize the risk of stroke. Fish product has a nutrient profile superior compare with other food sources like meats of beef, chicken and many more. Fish tissue possesses high nutritional value and is therefore a particularly recommended dietary component. The main constituent of fresh fish is water, which usually accounts for about 80% of the weight of a fresh white fish fillet. But, for dried fish, there are more than half of the water content will be lost. Moisture or water compound is important diluents of the nutrients in foodstuffs. Ash content is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals without a food. The three main types of procedure used to determine the ash content of foods are based on principles like dry ashing, wet ashing and low temperature plasma dry ashing (Julian, 2003). Fibre includes substances like chitin, cellulose and lignin. Many fish do not have enzyme cellulose which is necessary for the digestion, and fibre usually is regarded as unavailable as an energy source. But, cellulose is produced by the gut bacteria of many types of fish, as is chitin's in crustacean, and herbivorous fish are able to digest fibre. Fish product is an excellent sources of high quality animal protein and suitable for supplementary diets of carbohydrates content. The carbohydrate most commonly found in fish is starch which is a polymer of glucose. Glucose in carbohydrates is mainly acts as energy yielding compound and the major fuel of the tissue, constitutes the structural material of the organism. It involved in amino acids and fatty acids metabolism. Monosaccharide is very important to constitute nucleotides and nucleic acids while disaccharides involved in digestion and the major source of energy in the diet (Mohd, 2010).

Most fibrous protein plays structural roles in skin, connective tissue of fiber like wool and hair. Attention has been focused due to the consumption related to reduction of incidence in cardiovascular disease. It mainly due to the fat content in fish which is supplies omega-3 polyunsaturated fatty acids (PUFA). PUFAs are essential in lowering blood cholesterol level and high blood pressure. It takes role to migrate to alleviate platelet of cholesterol aggregation and atherosclerosis conditions in adult population. This function help to prevent some of the diseases like asthma, arthritis and some types of cancer (Tee *et al.*, 1987). It reduces the risk of sudden death from heart attack and reduces rheumatoid arthritis. Omega-3 fatty acid also lower the risk age related muscular degeneration and vision impairment, decrease the risk of bowel cancer, and reduce insulin resistance in skeletal muscle. High digestibility of fish lipids shows that it can provide usable energy. If a normal diet does not provide enough energy, fish as diet to break down valuable protein for energy, which is expensive and at the same time increasing production of nitrogenous compounds (Abbas *et al.*, 2009). Other than that, fish product also contains minerals like calcium and phosphorus. Many minerals are required in trace amounts and are present in sufficient quantity in water to absorb through their gills (Hassaan *et al.*, 2013). In body fluids they are involved mainly with the maintenance of osmotic equilibrium with the aquatic environment and the nervous and endocrine system; they

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are components of enzymes, blood pigments and other organic compounds. They provide strength and rigidity to bones in fish (Marian *et al.*, 2010). Both calcium and phosphorus are very important for strengthening bones and teeth. Calcium fortified foods like fishes are like to play an important role to help in maintaining the intake of calcium. Calcium able to activates ATP during muscular contraction to help in capillary permeability and blood clotting condition. For phosphorus, it involves in synthesis of nucleic acid, ATP and some protein. Phosphorus works with calcium to build bone. When calcium and phosphorus are out of balance, body system may run the risk of bone tissue forming in heart, liver and blood vessels. For salt content, high intakes will cause the calcium faster loss from human body. Normally there are huge amount of salt present in dried fish preserving process (Hassaan *et al.*, 2013).

The aim and objective of this study is to evaluate the proximate composition of three available marine dry fishes which is consumed by the Malaysian population. It is to evaluate the content are Ash, Moisture, Fibre, Salt, Total carbohydrates, Total protein, Total fat, Calcium and Phosphorus. This study focussed to compare the selected biochemical constituents listed above from the 3 different dried fishes namely *Rastrelliger kanagurta, Megalaspis cordyla* and *Otolithes ruber* bought from two different places of Kuala Lumpur market which are commonly consumed by the Malaysian people.

Materials and methods

Chemicals: The following major chemicals and the company produced were given in the parenthesis. Amino naphthol sulphanilic acid (ANSA), Ammonium molydbate, Copper sulphate, Folin Ciocalteau reagent, Glycerol, Ninhydrin reagent, Potassium thio-cyanate, Stannous Chloride, Sodium sulphite, and Silver nitrate (HiMedia Laboratories Pvt. Ltd.). Disodium hydrogen phosphate, Sulphuric acid, Nitric acid, Oxalic acid and liquid ammonia (R&M Marketing, Essex, UK). Ammonium oxalate and Chloroform (Systerm Classic Chemicals Sdn Bhd). Dipotassium hydrogen orthophosphate, Potassium Chloride and Sodium hydroxide (Bendosen), Methanol (Riendemann Chmidt). Sodium carbonate and Sodium Chloride (J Kollin Corporation, Germany), Sodium Potassium Tartrate (HmbG Chemical, Germany). 3,5 – dinitrosalicyclic acid (DNS) (Sigma Aldrich Ltd.) and Potassium dihydrogen phosphate (Merck Chemicals Co., Ltd.).

Estimation of Ash: About 2 g of the each dried fish sample was taken into a pre-heated crucible. The crucible was placed in muffle furnace at 400°C for 4 hours or until a whitish-grey ash was obtained; and then was placed in the desiccator and weighed. The percentage of ash content was calculated by using the following formula.

$$\% \text{ ash} = \frac{\text{weight of crucible+ash} - \text{weight of crucible}}{\text{weight of sample}} \times 100$$

Estimation of Moisture: The principle behind is that the moisture by the reduction in weight when the sample was dried to a constant weight in an oven. About 2 g of each fish sample was weighed and placed in an aluminium dish which has been previously dried and weighed; the sample was then dried again in an oven at 65°C for 36 hours, cooled in desiccator and weighed again. The process was continuously repeated until a constant weight was achieved. The following equation was used to determine the moisture content of the dry fish sample.

$$\% \text{ of moisture} = \frac{\text{weight of sample} - \text{weight of dried sample}}{\text{weight of sample taken}} \times 100$$

Estimation of Fibre: Petroleum ether was added to the 2 g of each sample to extract the fat, stirred and allowed to settle and decanted. The process was repeated for 3 times then transfer fat-free material into a flask. About 200 mL of pre-heated 1.25% H_2SO_4 was added and the solution was boiled for about 30 minutes. Hot water was added to maintain constant volume of acid. The boiled acid sample mixture was filtered through the funnel under sufficient section. The residue was washed several times with boiling water until it became neutral to litmus paper then transferred back into beaker. About 200 mL of pre-heated 1.25% Na_2SO_4 was added and boiled for another 30minutes, filter under section and wash thoroughly with hot water and twice with ethanol. The residue was dried at 65°C for 24 hours. The residue was weighed and transferred into a crucible then placed in muffle furnace (400–600°C) for 4 hours. The residue was allowed to cool in desiccator and weighed again. The following formula was used to calculate the percentage of fibre content present on the sample.

$$\% \text{ fibre} = \frac{\text{dry weight of residue (before} - \text{after ashing)}}{\text{weight of sample}} \times 100$$

Estimation of Salt: About 2 g of each sample was taken in a 250 mL beaker and 50 mL of distilled water free from chloride was added then heated on a water bath till all the Sodium Chloride goes into solution. The solution was filtered in a 250 mL conical flask and washed with distilled water till the washing were free from chloride. About 20 mL of 0.1 M nitric acid then added to the solution and a known volume of standard silver nitrate sufficient to precipitate all the chloride. About 1 mL of ferric alum indicator was added and titrated with standard Potassium thio-cyanate solution until a permanent light brown colour appeared.

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Estimation of Total Carbohydrates: About 2 g of each sample was taken and homogenized with 0.1 M phosphate buffered saline solution. The working standard solution was prepared to find the unknown samples concentration. To 1 mL of the homogenized solution, 3 mL of DNS solution was added and then placed in a boiling water bath for 5 minutes. The tubes were cooled thoroughly and added 7 mL of distilled water to each tube. The absorbance value was taken at 540 nm by using the solution in tube 1 as blank.

Estimation of Total Protein: About 2 g of each sample was taken and homogenized with phosphate buffer saline solution. About 6 ml of sample was added with 30 mL alkaline copper sulphate solution and mixed well then incubated for 5 minutes in room temperature. After the tubes have been allowed to stand, 3 mL of Folin-Ciocalteau reagent was added and mixed the solution rapidly. The tubes then left for 30 minutes in room temperature. The sample solution then was diluted to 1/5, 1/10, 1/50, 1/100 and 1/500 and the concentration was calculated. A blank was conducted by taking 1ml of distilled water then mixed with 5 mL of alkaline copper sulphate solution and 0.5 mL of Folin-Ciocalteau reagent. The absorbance value was taken at 750 nm.

Estimation of Total Fat: About 2 g of powdered dry tissue was mixed into 10 mL solution of chloroform and methanol in the ratio 1:2, and stirred with a glass rod. The resultant mixture then left over night and centrifuged in next day. After centrifuged, the clear supernatant was removed carefully into washed, dried and pre-weighed small bottles. These bottles were placed in an oven at 45°C to evaporate the solvent leaving the lipid fraction. The solvent was allowed to cool in desiccator and weighed. The percentage of fat was calculated using the following formula.

$$\% \text{ of fat} = \frac{\text{weight of flask+extract} - \text{tare weight of flask}}{\text{weight of sample}} \times 100$$

Estimation of Calcium: About 2 g of sample was mixed with 2 mL of distilled water then add with 1 mL of ammonium oxalate solution in each tube. The solution was mixed well and incubated for 30 minutes at room temperature. Then, the solution was centrifuged for 10 minutes and the supernatant was removed carefully without loss of precipitate. About 3 mL of liquid ammonia was added into the solution and centrifuged and again decanted the supernatant. It was washed with 3 mL of distilled water and the supernatant again was removed before and after centrifuging. About 2.0 mL of 1N sulphuric acid was added to the precipitate along the sides rotating the tubes which to make sure the precipitate was washed down to the bottom. The tube was placed in 70°C water bath. The calcium oxalate dissolved and the hot solution was titrated against the standardized (earlier) potassium permanganate till the end point was reached which have the appearance of pale pink colour. A blank was conducted by taking 2.0 mL of 1N of sulphuric acid, heating and titrating against the standardized permanganate, until the end point was reached. The readings were tabulated and calculated.

Estimation of Phosphorus: About 2 g of each sample was homogenized in phosphate buffer saline then diluted to 1/5, 1/10, 1/50 and 1/100 with distilled water. After that, 200 µL of ammonium molybdate and 30 µL of stannous chloride were added to each tube and mixed well. A blank was conducted by taking 5 mL of distilled water then mixed with 200 µL of ammonium molybdate and 30 µL of Stannous Chloride. The absorbance value was taken at 690 nm.

Statistical analysis: All the parameters had done in triplicates and the values were expressed as mean±SD. In Figure the whole numbers were taken to represent as percentage. In Tables, the values were given as such with mean±SD.

Results and discussion

Estimation of Ash, Moisture and Fibre content: In ash and moisture test, *Megalaspis cordyla* contained the highest value especially bought from Taman EngAnn which was 7.44% and 32.46% when compared with the dried fish bought from Jalan Meru which the values were 7.22% and 30.96% respectively (Fig. 1 and 2). *Otolithes ruber* had the lowest percentage in both ash and moisture test also but sample bought from Jalan Meru lower than those bought from Taman EngAnn. In ash estimation and for moisture test, the sequence was opposite which the sample bought from Taman EngAnn lower than those bought from Jalan Meru. Overall the values did not show significant in terms of buying places and this might be due to factors discussed below. Since the drying procedure is almost common, for dried fish, there are more than half of the water content was lost. Sun-dried fish contained more residual moisture than smoke-dried fish. This was because contrary to sun-dried fish which tends to moisturize ambient air humidity was high then the protective coating would reduce rehydration of smoke-dried. In the dried fish, total ash contents become higher due to the loss of water component (Ali *et al*., 2011). Increase in moisture content could be attributed to the difference in the moisture of the processed fish relative to the surroundings (Daramola *et al*., 2007). The fibre content was not so significant but little variation in terms of percentage was observed between the 2 places where we bought.

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Fig. 1. Ash, moisture and fibre content of the selected dry fishes bought from Taman EngAnn.

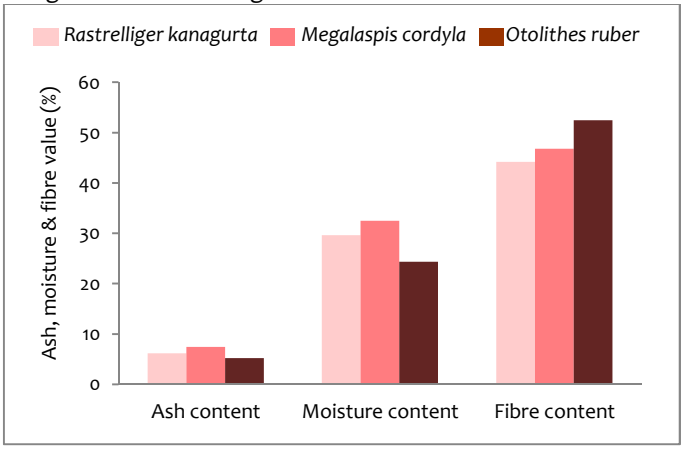

Fig. 2. Ash, moisture and fibre content of the selected dry fishes bought from Jalan Meru.

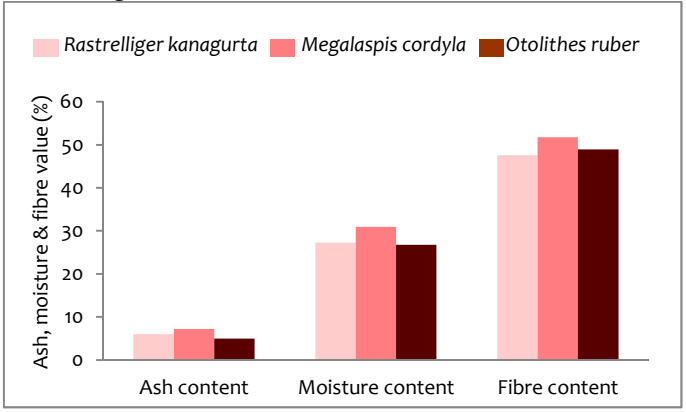

Table 1. Salt content (%) of fishes expressed as mean ± SD.

Dried fish variety	Taman EngAnn	Jalan Meru
Rastrelliger kanagurta	27.40 ± 0.68	25.67 ± 0.83
Megalaspis cordyla	29.38 ± 1.17	26.45 ± 0.91
Otolithes ruber	20.16 ± 0.77	23.86 ± 1.42

The increase in the crude fibre content could be accounted for by the present of oxidation in poly-unsaturated fatty acids (PUFA) components, contained in their tissues to products such as peroxides, aldehydes, ketones and free fatty acids (Daramola *et al*., 2007). Increased of crude fibre content of the fish may due to the increase of protein component. Fibre may cause reduced protein utilization then make the difference between adequacy and inadequacy of proteins.

Estimation of Salt content: *Megalaspis cordyla bought* from 2 different places contained maximum salt content with 29.38% and 26.45% respectively (Table 1). Combination of drying with salt, fish can be preserved and creating an environment inhospitable for bacteria when salt content increasing. This is because salt can kill the bacteria by dehydrating them. But, high intakes of salt will cause the increase the rate of calcium loss from human body. Sodium contains in salt helps muscles and nerves work properly by assisting muscular contraction and transmission of nerve signals. But, overtake of salt promotes urinary calcium loss and have the potential to cause bone mineral deficits if intestinal calcium absorption does not compensate for these losses (Navidi *et al*., 1995).

Estimation of Total Carbohydrates and Total Protein: The results of total carbohydrates and total protein content were expressed in g per 100g of sample. *Megalaspis cordyla* bought from Taman EngAnn and Jalan Meru was containing the highest amount in protein test which were 11.48 g and 11.20 g respectively per 100g of sample (Fig. 3 and 4). Increase of protein may be due to the dehydration of water molecule present between the proteins thereby, causing aggregation of protein and thus resulting in the increase in protein content of sample (Oladipo and Bankole, 2013). Ogbonnaya and Shaba (2009) reported that protein nitrogen was not lost during drying, thus protein content increasing when the moisture content become lower in fish sample. When the fish temperature is kept below 70°C during process, the drying time and heat affect the protein quality. Thus, the methods of fish drying may affect the protein content. For carbohydrates, *Rastrelliger kanagurta* from Taman EngAnn presented the highest value which was 6.86 g while *Megalaspis cordyla* from Taman EngAnn contained the lowest carbohydrate content which was 5.2 g per 100g of sample. Presence of carbohydrate and protein content in dried fish normally is lower than fresh fish due to the prevalence of unhygienic conditions, improper handling and inadequate preservation (Patterson and Ranjintha, 2009). According to Emokpae (1980), observed changes in protein and lipid content during storage may have been due to leaching out of some extractable soluble protein fraction and hydrolysis of some of the lipids fractions. Other than that, glycogen which not contributed much in marine animals like fish may cause low values of carbohydrates in general (Daramola *et al*., 2007). Total fat in *Megalaspis cordyla* bought from Jalan Meru was comparatively high in percentage (17.82%) but they were not significant. According Ogbonnaya and Shaba (2009), increase of fat content after fish drying could be result of evaporation of moisture or water molecule present. Reduction in crude fat content could have been due to oxidation (Ali *et al*., 2011). The greater the degree of unsaturation, the greater would be the tendency for fat oxidation. Higher fat also can be observed in ripe and gravid fish where a low level of fat was recorded in young fish. Seasonal differences in the availability of food and changes in the reproduction cycle have considerable effect on tissue biochemistry of the fish, especially for fat component.

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Fig. 3. Total Carbohydrate and Protein content of the selected dry fishes bought from Taman EngAnn.

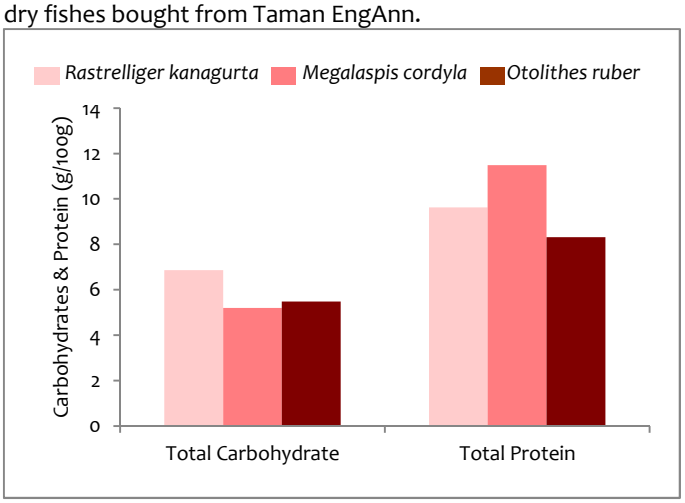

Fig. 4. Total Carbohydrate and Protein content of the selected dry fishes bought from Jalan Meru.

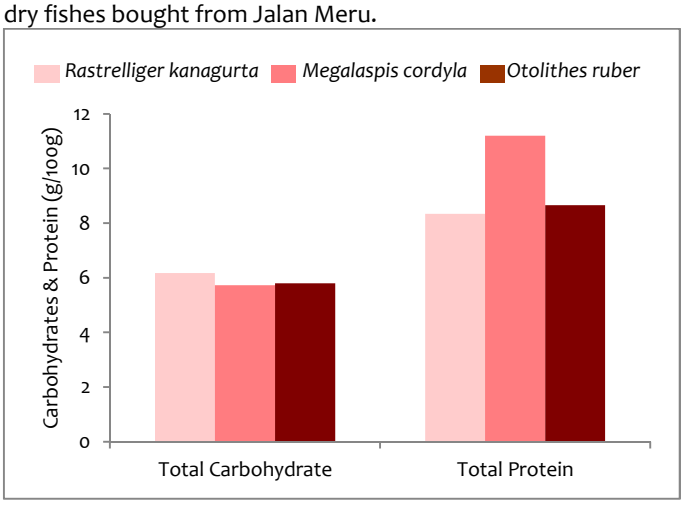

Fig. 5. Calcium and Phosphorus content of the selected dry fishes bought from Taman EngAnn.

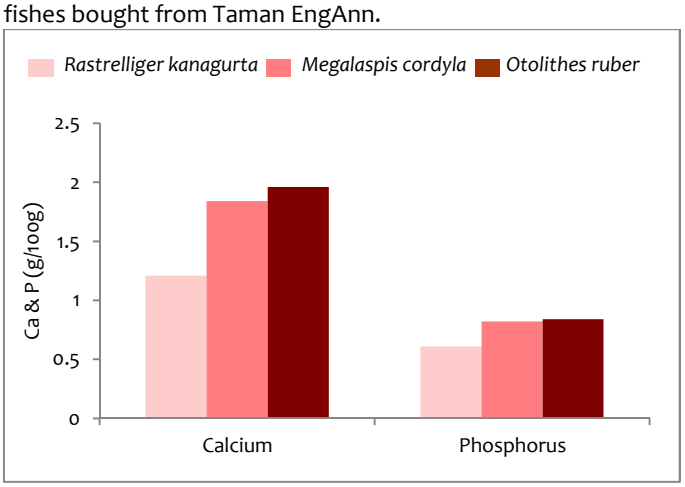

Fig. 6. Calcium and Phosphorus content of the selected dry fishes bought from Jalan Meru.

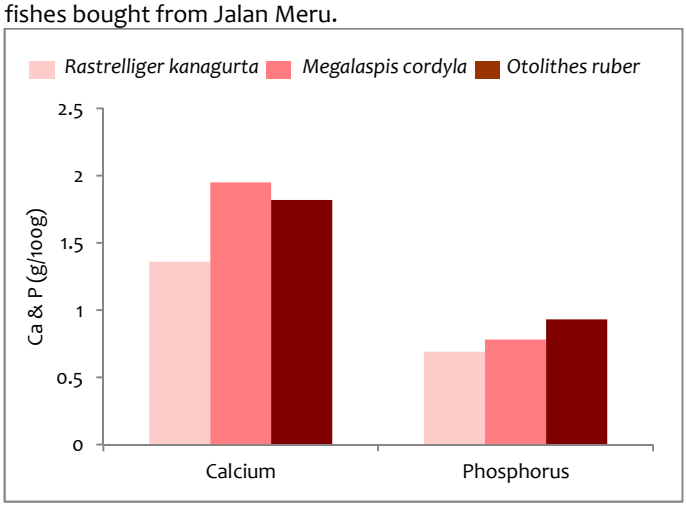

Table 2. Total fat content expressed as mean ± SD.

Dried fish variety	Taman EngAnn	Jalan Meru
Rastrelliger kanagurta	13.40 ± 1.21	14.36 ± 1.89
Megalaspis cordyla	16.62 ± 1.48	17.82 ± 1.70
Otolithes ruber	14.30 ± 1.06	15.34 ± 1.28

The greatest concentrations of fat can be found at the end of the prolific feeding in summer and the least in winter (Table 2). The percentage of water is a good indicator of its relative contents of energy, proteins and lipids. The lower percentage of water would be greater the lipids and protein content and higher the energy density of the fish. However, these values vary considerably within and between species, size, physical activity and many more.

Estimation of Calcium and Phosphorus content: In calcium test, *Otolithes ruber* from Taman EngAnn contained 1.96 g was slightly higher than *Megalaspis cordyla* bought from Jalan Meru (1.95 g/100 g of sample) (Fig. 5 and 6). *Otolithes ruber* bought from Taman EngAnn was the highest value of phosphorus content (0.84 g/100 g of sample) while *Rastrelliger kanagurta* collected from Taman EngAnn too having lowest value which was 0.61 g/100 g. Several studies have indicated that calcium and phosphorus can be absorbed and excreted through the gill membranes of fish. Thus, dietary requirements are probably affected by the water levels of these nutrients (Stanek *et al.*, 2013). A study on calcium and phosphorus of dried fish stated that usually in small fishes minerals comprise about 2% of the whole fish (Marian *et al.*, 2010).

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Conclusion

The proximate composition of 3 different marine dry fishes revealed that dry fish have very good nutritional value. But, longer storage of dry fishes is greatly deteriorates the food nutritional values. Other than that, the methods used for fish drying and the catching period also affect the nutritional values on fish. This is due to the seasonal differences and changes in the reproduction cycle of fishes have considerable effect on tissue biochemistry. So, the test can be more detail by differentiate the methods involved in fish drying. From the comparison of the nutritional values in 3 varieties of fishes *Megalaspis cordyla* had significantly high for 7 among 9 parameters. The 7 parameters were ash, moisture, salt, protein, carbohydrates, calcium and phosphorus. For *Rastrelliger kanagurta*, it contained highest value for carbohydrates which was 6.86 g while fibre content of *Otolithes ruber* presented the highest percentage which was 52.46%. In general, these two varieties of fishes having the almost close result in the remaining parameters. In overall, the two different places we bought the sample were having the close result on compared for the 9 biochemical parameters for its composition.

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