



**Investigation of Proximate Composition in Two Different Treatment
of Asian clams (*Corbicula fluminea*) at Selected District in Kelantan**

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**A report submitted in fulfilment of the requirement for the degree of
Bachelor of Applied Science (Animal Husbandry) with Honours**

Faculty of Agro Based Industry

UNIVERSITI MALAYSIA KELANTAN

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

Student

Name:

Date:

I certify that the report of this final year project entitled Investigation of Proximate Composition in Two Different Treatment of Asian clams (*Corbicula fluminea*) at Selected District in Kelantan by Musni Suryani binti Sarazudin, matric number F15A0094 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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Investigation of Proximate Composition of Asian clams (*Corbicula fluminea*) in Two Different Treatment at Selected District in Kelantan

ABSTRACT

This research investigated the proximate composition of Asian clams (*Corbicula fluminea*) in two different treatment which are fresh and smoked at selected district in Kelantan which are Pasir Mas and Tumpat. Five components in proximate composition (moisture, ash, crude fat, crude protein and carbohydrate) were evaluated. The moisture contents of the fresh samples were higher compare to the moisture of the smoked samples. The results showed that the crude protein, crude fat and ash contents of the smoked samples from both harvested location were higher compare to the fresh samples. However, there are some error occur and the value of carbohydrate from both treatment and both harvested location might not be accurate. The value of moisture content, ash content, crude fat content, crude protein content and carbohydrate content between both treatment are not significantly different ($p>0.05$). Thus, the result shows that, smoking process help in increase the value of proximate composition.

Keywords : *Corbicula fluminea*, ash, crude fat, crude protein, smoking

Penyiasatan Komposisi Proksimat kerang Asia (*Corbicula fluminea*) dalam Dua Rawatan Berbeza di Daerah Terpilih di Kelantan

ABSTRAK

Kajian ini menyelidik komposisi kerang Asia (*Corbicula fluminea*) dalam dua jenis rawatan iaitu kerang segar dan kerang salai di daerah terpilih di Kelantan iaitu Pasir Mas dan Tumpat. Lima komponen dalam komposisi proksim (kelembapan, abu, lemak mentah, protein mentah dan karbohidrat) telah dinilai. Kandungan kelembapan sampel segar lebih tinggi berbanding kelembapan sampel salai. Keputusan menunjukkan bahawa protein mentah, lemak mentah dan kandungan abu dari sampel yang dinilai dari kedua-dua daerah adalah lebih tinggi berbanding sampel segar. Walau bagaimanapun, terdapat beberapa kesilapan berlaku dan nilai karbohidrat dari kedua-dua rawatan dan kedua-dua daerah mungkin tidak tepat. Nilai kandungan kelembapan, kandungan abu, kandungan lemak mentah, kandungan protein mentah dan kandungan karbohidrat antara kedua-dua rawatan tidak jauh berbeza ($p > 0.05$). Oleh itu, hasilnya menunjukkan bahawa, proses dapat membantu meningkatkan nilai komposisi proksim.

Kata kunci : *Corbicula fluminea*, abu, lemak mentah, protein mentah, proses salai

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LIST OF ABBRECIATION

<i>sp.</i>	Species
ANOVA	Analysis of variance
SD	Standard deviation
CP	Crud protein
CF	Crude fat
NFE	Nitrogen free extract



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LIST OF SYMBOLS



μl	microliter
$^{\circ}\text{C}$	degree celsius
%	percentage
cm	centimetre
g	gram
m	meter
ml	milliliter
mm	millimeter
mg	milligram
μg	microgram
h	hour

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Corbicula fluminea known as a filter feeder that removes particles. According to McMahon (1991), a small light-colored bivalve with shell ornamented by distinct, concentric sulcations, anterior and posterior lateral teeth with many fine serrations. In southwestern United States dark shell morphs exist but are limited. There are two types of shell morph which has two different colour. The light shell morph has a yellow-green to light brown periostracum and white to light blue or light purple nacre while the darker shell morph has a dark olive green to black periostracum and deep royal blue nacre.



Figure 1.1 Shell of *Corbicula fluminea*

The Asiatic clam is found throughout Asia, North and South America, Europe and parts of Africa (Robert Naumann, n.d). According to Jueg and Zettle (2004), the taxonomy of *Corbicula fluminea* is start from kingdom. The kingdom of *C.fluminea* is Animalia the phylum is *Mollusca*, the class is *Bivalvia*, the subclass is *Heterodonta*, the order is *Veneroida*, the suborder is *Sphaeriacea*, the superfamily is *Corbiculoidea*, the family is *Corbiculidae*, the genus is *Corbicula* and species is *Corbicula fluminea*. Figure 1.1 is the image of *Corbicula fluminea* shell.



Figure 1.2 Smoked *Corbicula fluminea*



Figure 1.3 : Roadside of Tumpat

Asian Clam is an ecologically and economically important freshwater shellfish in South-East Asian region especially in Malaysia and Thailand (Zalina Che Manan, 2014). Several states in Malaysia such as Kelantan, Terengganu, Perak, and Pahang have the most numerous harvested freshwater shellfish for consumption in. In general, although it is least known or simply neglected by most Malaysians there is growing demand for Asian clams in Malaysian markets especially in Kelantan. In Kelantan, Asian Clam or known as “Etok” is one of the most popular foods especially the smoked Asian Clam, where Kelantanese treated

it as a favorite pastime snacks (Figure 1.2). Smoked Asian Clam can be purchased in many places like the markets, night markets, and even at roadside Pasir Mas and Tumpat district areas (Figure 1.3). Therefore, the Asian Clam industry offers great potential to the state in terms of increasing the income of small-scale fishermen faced with dwindling catches. Since Asian Clam has become special snack for the Kelantanese, therefore only people in this state know how to prepare and consume it.

Traditionally, the Asian Clam industry in Kelantan has been carried out on a small-scale basis of enterprise. According to the local smoked Asian Clam vendors, demands of this species keep increasing over the years. Since Asian Clams in Kelantan are exploited due to sand mining project all along the Kelantan's main river, most of commercialized Asian Clams were imported from Pahang, Terengganu, and even Thailand by local vendors. Smoked Asian Clam is considered as an appetizer because it is light yet delicious meal to start.

Based on the chemical properties of the compounds, proximate analysis can be partitioning into six categories of compound in a feed and the six categories are crude fat, crude protein, moisture, ash, and crude fibre(digestible carbohydrates) (Károly Dublicz 2011). At 100°C, the loss in weight that results from drying a known weight of food to constant weight is known as moisture. The ash content is determined by ignition of a known weight of the food at 550°C until all carbon has been removed. Over 100 years ago, a modification of a technique originally devised by Kjeldahl was introduced to calculate the crude protein (CP). A continuous extraction with petroleum ether for a defined period can lead to determination of crude fat (CF). The carbohydrate of the food is contained in two fractions, the crude fibre (CF) and the nitrogen-free extractives (NFE). The former is

determined by subjecting the residual food from ether extraction to successive treatments with boiling acid and alkali of defined concentration the organic residue is the crude fibre.

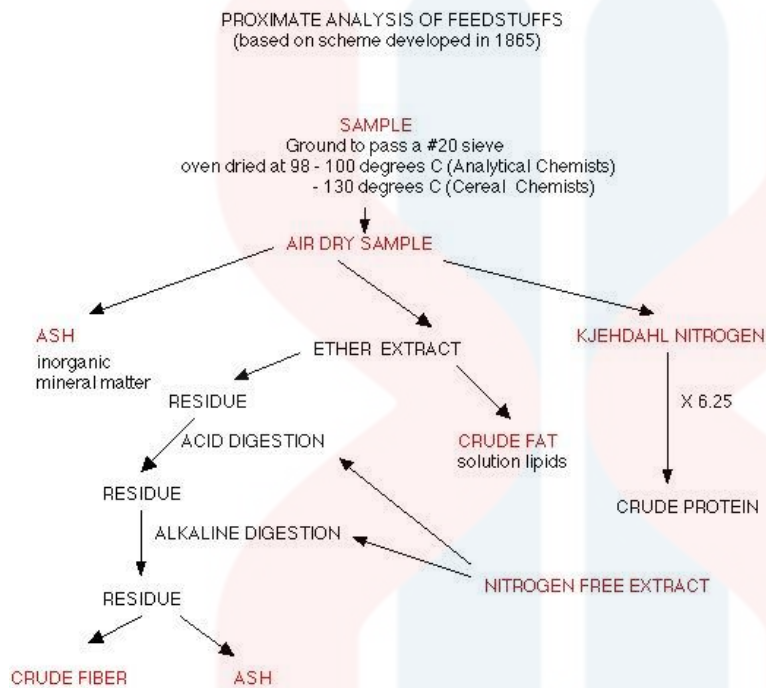


Figure 1.4 : Flow chart of proximate analysis

1.2 Problem Statement

There is different between the value of nutrition in fresh *Corbicula fluminea* and smoked *Corbicula fluminea*. As we know, there are several factor that affect the loss of nutritional value in *Corbicula fluminea*. This proposed research is to find the specific nutritional value of *Corbicula fluminea*, to find the nutritional value of *Corbicula fluminea* between two types of treatment and to analyze the relationship between harvested location and nutritional value of *Corbicula fluminea*. Approximate analysis is use in this research to find the nutritional value of *Corbicula fluminea*.

1.2 Hypothesis

The main hypothesis in this research is :

H_a - The nutritional value of *Corbicula fluminea* between two types of treatment are not significantly different.

H_o - The nutritional value of *Corbicula fluminea* between two types of treatment are significantly different.

1.3 Objectives

There are three objectives in this research which are

1. To determine the nutritional value of *Corbicula fluminea* in different harvested location.
2. To identify the nutritional value of *Corbicula fluminea* between two types of treatment.
3. To analyze the relationship between harvested location and nutritional value of *Corbicula fluminea*.

1.5 Scope of Study

This study was conducted in the Universiti Malaysia Kelantan Jeli Campus where the samples were collected from two selected region in Kelantan which are from Tumpat and Pasir Mas. In the proposed study the nutritional value of the *Corbicula fluminea* from the selected regions were investigated. From this two regions, the nutritional value of *Corbicula fluminea* between two types of treatments were observed. The two types of treatments were; fresh and smoked *Corbicula fluminea*. Other than that, the relationship between the selected regions which are Tumpat and Pasir Mas and the nutritional value of the *Corbicula fluminea* were analyzed.

1.6 Significance of Study

This study is significant for determining the nutritional value of *Corbicula fluminea*. The finding of this study will reveal the nutritional value of the *Corbicula fluminea* which mean, it will generate the information about the nutritional value of *Corbicula fluminea* to the consumers. Next, this study will determine the nutritional value between two types of treatments of *Corbicula fluminea* which are fresh and smoked. This study will acknowledge people on which type of treatment will have the highest nutrition content between the two types of treatment. This study can be applied as a reference to the consumers which one of the *Corbicula fluminea* from the selected regions have the highest nutritional value

CHAPTER 2

LITERATURE REVIEW

2.1 Origin and Spread

The Asian Clam *Corbicula fluminea* known as a native clams towards fresh waters of eastern and southern Asia. *Corbicula fluminea* is considered as one of the most important non-native invasive species (NIS) in aquatic systems due to its widespread distribution and ecological and economic impacts. It was likely introduced to the West Coast of North America around 1930, initially assumed to have been imported as a food source for the immigrating Chinese population (USACE ERDC, 2007). “In terms of both abundance and biomass, invasive bivalves of the genus *Corbicula* (Asian clam) are one of the most widespread species” (Juan Carlos, 2008).

The actual distribution of the Asian clam includes southeastern and eastern Asia, Australia and Africa (McMahon, 1983). The species *Corbicula fluminea* (Müller, 1744) and *Corbicula fluminalis* (Müller, 1744) are among the most “efficient” freshwater invaders worldwide, appearing as exogenous species in watercourses of both North and South America, Japan and Europe (Araujo et al., 1993). The IUCN Global Invasive Species

database considers the second species as synonym of the first, so hereafter both species will be considered as *Corbicula fluminea*.

2.2 Biology and Ecology

2.2.1 Reproductive Biology

Similar mode of reproduction in *C. fluminea*, *C. fluminalis*, *C. leana* and *C. australis* are suggest in recent investigation (Korniushin, 2004). This genus exhibits a wide variety of reproductive strategies, involving sexually reproducing species with both sexes or hermaphrodites (Korniushin & Glaubrecht, 2003)

All freshwater species in the genus *Corbicula* should be considered clonal lineages (Siripattrawan, Park, & Foighil, 2000). However, this does not apply in European populations since here morphotypes assigned to *C. fluminea* are meiotic and capable of hybridization with *C. fluminalis* (Pfenninger et al., 2002). Correlation with spermatozoa morphology and reproductive mode was characterized in *Corbicula* by biflagellated sperm, which are considered a marker for androgenesis, and presence of monoflagellated spermatozoa, indicating sexual reproduction (Ishibashi et al., 2003).

Spawning can occur throughout the year at water temperatures of 16°C or higher and fertilization occurs inside the paleal cavity and larvae are incubated in the gills. Larvae can be densely packed in the interlamellar space or irregularly distributed. Reproductive forms

with androgenesis have been recorded in *C.fluminea* (Korniushin, 2004) .Fecundation occurs and the oocyte ejects the entire maternal nuclear genome as two polar bodies (Ishibashi et al., 2003).The descendents remain with the paternal genetic information, but retains the mitochondria. However, Hedtke et al. (2008), analysing androgenic lineages of *Corbicula sp.* in the American continent, found mtDNA contamination corroborating earlier findings by Lee et al. (2005), and gives the egg parasitism process as the probable explanation for disruption in mtDNA lineages.

2.2.2 Environmental Requirements

For *C. fluminea*, ideal sediments are sand mixed with silt and clay, while rocky and pure silt exclude this species especially if the concentration of oxygen is low (Karatayev, Burlakova, T. Kesterson, & Padilla, n.d.). *C. fluminea* inhabits by decreasing order of preference such as fine sand, organically-enriched fine sand, coarse sand. However, it can also inhabit a vast variety of substrata, from fine sand to gravel. Concerning water levels, when *Corbicula* is exposed to low water levels long migration it can decrease population size. On the other hand, the increase in suspended sediments in the water column can cause mortality to *C. fluminea* in all age classes.

Even though there are no available data for pH limits for *C. fluminalis* or *C. fluminea*, lower pH values can increase the rates of mortality. “Asian clams were reported to be dying over 3 year period due to pH lower than 5.6 in Mosquito Creek in Florida” (Karatayev, Padilla, Minchin, Boltovskoy, & Burlakova, 2007). For short periods, *C. fluminea* is able to

tolerate salinities of up to 13 ppt and able to tolerate up to 24 ppt salinity. It has been reported in brackish and estuarine habitats even it generally known to occur in freshwater bodies.

“There are no data on *C. fluminalis* or *C. fluminea* concerning oxygen, calcium or upper and lower temperature limits” (Karatayev et al., 2007). Nevertheless, in *C. fluminea* low dissolved oxygen inhibits growth and high temperatures cause mass mortalities and declines in body mass (Vohmann et al., 2010).

Table 2.1 : Maximum of salinity and water temperature for *Corbicula*. (AODC 2009)

Parameter	Minimum Value	Maximum Value	Typical Value	Status	Life Stage	Notes
Salinity (part per thousand)		13		Optimum	Adult	24 ppt can be tolerated if acclimated
Water temperature (°C temperature)	2	30		Optimum	Adult	0°C tolerated

2.3 Proximate Analysis

Proximate analysis is a chemical scheme of feed analysis designed to measure the major nutrients in livestock feeds that developed in Germany by Henneberg and Stohman the late 1860s. In proximate analysis, there a few categories based on chemical properties that be measured such ash moisture, ash, crude protein, crude fat and crude fiber.

2.3.1 Moisture

The taste, texture, weight, appearance, and shelf life of foodstuffs influences by moisture content (*Food quality and safety.*, n.d.). Even a slight deviation from a defined standard can adversely impact the physical properties of a food material such as substances which are too dry could affect the consistency of the end product. During production, excess moisture may cause food material to agglomerate or become trapped in the piping systems. The resulting in spoiled batches that need to be disposed may cause by the rate of microbial growth increases. However, water is also an inexpensive ingredient adding to the weight of the final product.

Methods and procedures for moisture analysis are important since the method used to determine moisture may lead to varying results for moisture content, depending on the form of the water present in a food. The drying oven, commonly used for commercial purposes, is the established reference method for loss on drying. In this procedure, a sample is weighed and subsequently heated to allow for the release of moisture. Following this, the sample is cooled in the desiccator before reweighing. Moisture content is calculated by the difference in wet and dry weight. In this process, measuring accuracy and the resolution of the balance are extremely important. Careful consideration must also be given to maintain identical conditions, where temperature and duration are vital for generating precise and reproducible results.

2.3.2 Ash

Table 2.2 : Ash content in selected food.

<i>Food Item</i>	<i>Percent Ash (Wet Weight Basis)</i>
<i>Cereals, bread, and pasta Rice, brown, long-grain, raw</i>	<i>1.5</i>
<i>Corn meal, whole-grain, yellow</i>	<i>1.1</i>
<i>Hominy, canned, white</i>	<i>0.9</i>
<i>White rice, long-grain, regular, raw, enriched</i>	<i>0.6</i>
<i>Wheat flour, whole-grain</i>	<i>1.6</i>
<i>Macaroni, dry, enriched</i>	<i>0.9</i>
<i>Rye bread</i>	<i>2.5</i>
<i>Dairy products</i>	
<i>Milk, reduced fat, fluid, 2%</i>	<i>0.7</i>
<i>Evaporated milk, canned, with added vitamin A</i>	<i>1.6</i>
<i>Butter, with salt</i>	<i>2.1</i>
<i>Cream, fluid, half-and-half 0.7</i>	
<i>Margarine, hard, regular, soybean</i>	<i>2.0</i>
<i>Yogurt, plain, low fat</i>	<i>1.1</i>
<i>Fruits and vegetables</i>	
<i>Apples, raw, with skin</i>	<i>0.2</i>
<i>Bananas, raw</i>	<i>0.8</i>
<i>Cherries, sweet, raw</i>	<i>0.5</i>
<i>Raisins</i>	<i>1.9</i>
<i>Potatoes, raw, skin</i>	<i>1.6</i>
<i>Tomatoes, red, ripe, raw</i>	<i>0.5</i>
<i>Meat, poultry, and fish Eggs, whole, raw, fresh</i>	<i>0.9</i>
<i>Fish fillet, battered or breaded, and fried</i>	<i>2.5</i>
<i>Pork, fresh, leg (ham), whole, raw</i>	<i>0.9</i>
<i>Hamburger, regular, single patty, plain</i>	<i>1.9</i>
<i>Chicken, broilers or fryers, breast meat only, raw</i>	<i>1.0</i>
<i>Beef, chuck, arm pot roast, raw</i>	<i>1.1</i>

Table 2.2 is the example of food and the percent of ash content in the food. .

According to Nielson, (n.d). ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff To ensure reliable results, a basic knowledge of the characteristics of various ashing procedures and types of equipment is essential. There are two major types of ashing are used in the laboratory. The primary for

proximate composition is dry ashing and wet ashing (oxidation) is used for some types of specific mineral analyses, as a preparation for the analysis of certain minerals. Microwave systems now available for both dry and wet ashing to speed the processes. Most dry samples such as whole grain, cereals, and dried vegetables need no preparation, while fresh vegetables need to be dried prior to ashing. High-fat products such as meats may need to be dried and fat extracted before ashing. The ash content of foods can be expressed on either a wet weight (as is) or on a dry weight basis.

2.3.3 Crude Fat

By definition, lipids are soluble in organic solvents and insoluble in water. Therefore, water insolubility is the essential analytical property used as the basis for the separation of lipids from proteins, water, and carbohydrates in foods. Glycolipids are soluble in alcohols and have a low solubility in hexane. In contrast, triacylglycerols are soluble in hexane and petroleum ether, which are nonpolar solvents. The wide range of relative hydrophobicity of different lipids makes the selection of a single universal solvent impossible for lipid extraction of foods. Some lipids in foods are components of complex lipoproteins and liposaccharides. Therefore, successful extraction requires that bonds between lipids and proteins or carbohydrates be broken so that the lipids can be freed and solubilized in the extracting organic solvents.

According to Nielson, (n.d) the Soxhlet method (AOAC Method) is an example of the semi continuous extraction method. For semi continuous solvent extraction, the solvent

builds up in the extraction chamber for 5–10 min and completely surrounds the sample and then siphons back to the boiling flask. Fat content is measured by weight loss of the sample or by weight of the fat removed. This method provides a soaking effect of the sample and does not cause channeling. However, this method requires more time than the continuous method.

2.3.4 Crude Protein

Proteins can be classified by their biological function, composition, structure, or solubility properties. For example, simple proteins contain only amino acids upon hydrolysis, but conjugated proteins also contain non-amino-acid components. The analysis of proteins is complicated by the fact that some food components possess similar physicochemical properties. Numerous methods have been developed to measure protein content. The basic principles of these methods include the determinations of nitrogen, peptide bonds, aromatic amino acids, dye-binding capacity, ultraviolet absorptivity of proteins, and light scattering properties.

In the Kjeldahl procedure, proteins and other organic food components in a sample are digested with sulfuric acid in the presence of catalysts. The total organic nitrogen is converted to ammonium sulfate. The digest is neutralized with alkali and distilled into a boric acid solution. The borate anions formed are titrated with standardized acid, which is converted to nitrogen in the sample. The result of the analysis represents the crude protein content of the food since nitrogen also comes from non-protein components.

2.3.5 Carbohydrate

Carbohydrates are one of the most important components in many foods. Carbohydrates may be present as isolated molecules or they may be physically associated or chemically bound to other molecules. Individual molecules can be classified according to the number of monomers that they contain as monosaccharides, oligosaccharides or polysaccharides. Molecules in which the carbohydrates are covalently attached to proteins are known as glycoproteins, whereas those in which the carbohydrates are covalently attached to lipids are known as glycolipids. Some carbohydrates are digestible by humans and therefore provide an important source of energy, whereas others are indigestible and therefore do not provide energy. Indigestible carbohydrates form part of a group of substances known as dietary fiber, which also includes lignin.

A large number of analytical techniques have been developed to measure the total concentration and type of carbohydrates present in foods. The carbohydrate content of a food can be determined by calculating the percent remaining after all the other components have been measured. Nevertheless, this method can lead to erroneous results due to experimental errors in any of the other methods, and so it is usually better to directly measure the carbohydrate content for accurate measurements.

CHAPTER 3

METHODOLOGY

3.1 Sample Collection and Preparation

The sample were purchased early in the month of May, June and September from two district in Kelantan which are from Tumpat and Pasir Mas. The sample were purchased between two treatments from fresh and smoked of three different stall of selected place. The Asian clams (*Corbicula fluminea*) were prepared by separate the flesh from the shell and the flesh were stored in the petri dish. Meanwhile, about 100g of the flesh from each of the treatment and stall were dried in the dry oven for three days. The dried samples then were blended into powder and were stored in the petri dish according to their types. In the other hand, all the wet samples were stored in the freezer and were before further analysis.

3.2 Chemicals, Reagents and Equipment

Tablet kjeltab catalyst, concentrated sulfuric acid (HO_2SO_4), 4% boric acid (H_3BO_3), Green bromocresol, 40% Sodium hydroxide (NaOH), 0.1 N Hydrochloric acid, and Petroleum ether

Precision balance, Air-circulation oven, Desiccator, Crucible and lid, Grinder, Furnace, Tube, 250ml Conical flask, Filter funnel, Retort stand, Extraction thimbles, Round bottle flask, Soxhlet

3.3 Proximate Analysis

The proximate compositions were determined following the AOAC (2006) methods. Carbohydrate was calculated by using equation 3.7.

3.3.1. Determination of Moisture Content

All the crucible were dried in oven for 30 min. After dried, the crucibles were cooled in desiccator to avoid moisture absorption from surrounding. The sample was grounded first to make sure the uniformity of heat penetration. The cooled dried crucible initially weighed. 5g of ground sample was placed in the crucible. The crucible with the samples were dried at

105°C for 24 hours. After dried, crucible with the samples were cooled in the desiccator and reweighed. Percentage dried matter and moisture content were calculated using Equation 3.1 and Equation 3.2.

$$\text{DM (\%)} = \frac{\text{Weight of dried matter}}{\text{Weight of sample}} \times 100\% \quad (3.1)$$

$$\text{Moisture (\%)} = 100 - \% \text{ of dried matter} \quad (3.2)$$

3.3.2. Determination of Ash Content

The sample was grinded and was weighed about 5g. The dried crucible were weighed prior to sample loading. The samples in the crucible were placed into the Furnace at 550°C for 24 hours. Next day, the samples was taken out from the Furnace and was cooled in the desiccator. The final weight of the crucible with the sample will be measured using Equation 3.3.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Initial weight of sample}} \times 100\% \quad (3.3)$$

3.3.3 Determination of Crude Fat Content

All the glass apparatus were rinsed by petroleum ether, were dried at 102°C in the oven and were cooled in the desiccator. 5g of dried sample was weighed and was placed in the extraction thimble. 150ml round bottle flask was taken and was filled with 300ml of petroleum ether. The whole setting were placed on heating mantle and allowed the petroleum ether to boil at boiling temperature. The extraction process was continued almost 6 hours. The condensing unit was removed from extraction unit and allowed the sample to cool down. Almost all the solvent will be collected after distillation. The round bottle flask was placed in the oven and was dried at 102°C for 1-2 hours until constant weight reach. The round bottle flask was cooled in desiccator and was weighed by using Equation 3.4.

$$\text{Crude fat (\%)} = \frac{\text{Weight of flask with extract}}{\text{Weight empty flask}} \times \frac{100\%}{\text{weight of sample}} \quad (3.4)$$

3.3.4. Determination of Crude Protein Content

The protein was determined by using Kjeldahl method (Nielsen, 2010). The Kjeldahl method can be divided into three parts (1) Digestion, (2) Distillation, (3) Titration. The equipment used were Gerhardt Kjeldatherm and Gerhardt Vapodest.

3.3.4.1 Digestion

About 1.0g of wet sample was weighed and inserted into each digestion tubes. Each tube was added with two pieces tablets of Kjeltabs catalyst. Next, 12 mL of concentrated H_2SO_4 solution was added into each tube inside the fume chamber and slowly shaken. The tubes were connected to the digester. After 3 hours, when acid vapour appeared at exhaust system, the system will stop. Cooled the tubes vertically about 30 to 40 minutes. 80.0ml of distilled water and 50.0ml of 40% NaOH was poured into cold tube and continued for distilled process.

3.3.4.2 Distillation

For preparing receiver solution, 6.0g of 4% Boric acid, 2.1ml of methyl red indicator, 3.0ml of Green Bromocresol indicator and 300ml of distilled water were filled into 250ml conical flask. Receiver solution was placed on receiving platform. The sample was distilled by the following:

Dilution (distilled water)	80ml
Alkali	50ml
Receiver solution	30ml
Distillation time	4 minutes

3.2.4.3 Titration

The product from distillation process was titrated with 0.1 N HCl until the color turned to pale pink. Titration volume was recorded. Step 3 until step 13 were repeated without sample and were calculated by using Equation 3.5 and Equation 3.6.

$$\text{Nitrogen (\%)} = \frac{(T-B) \times N \times 14.007}{\text{Weight of sample(mg)}} \quad (3.5)$$

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25 \quad (3.6)$$

T = Sample titration

B = Blank titration

N = Normality of HCl

3.3.5 Determination of Carbohydrate Content

The percentage of carbohydrate was determined by the difference between the sum of the percentage of moisture, ash, crude protein and crude fat with 100. The formula is as shown in Equation 3.7

$$\text{Carbohydrate(\%)} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ Crude fat} + \% \text{ crude protein}) \quad (3.7)$$

3.3 Statistical Analysis

All of the extraction and composition analyses were conducted in triplicates from two independent experiments conducted for this study. Results were expressed as mean values \pm standard deviation (SD). The differences between the mean values of *Corbicula fluminea* fresh and smoked were calculated using two-way analysis of variance (ANOVA), and statistically significant differences were reported at $P < 0.05$. Data analyses were done with the use of SPSS 25.0 software.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Proximate Analysis

4.1.1 Moisture Content

Based on Table 4.1, the relationship between the moisture content of *Corbicula fluminea* in two different harvested location which are from Pasir Mas and Tumpat and the moisture content of *Corbicula fluminea* from different treatment which are fresh and smoked.

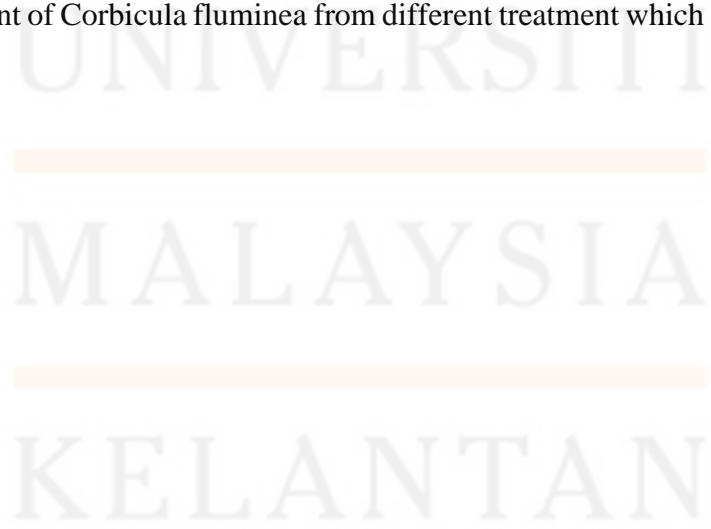


Table 4.1 : Moisture content (%) ± S.D for different treatment.

Harvested location / Fresh		Moisture Content (%) ± S.D.	
		Fresh	Smoked
Pasir Mas	PM1	80.5622 ± 0.50 ^a	79.0431 ± 0.73 ^a
	PM2	80.4273 ± 0.58 ^a	79.0642 ± 0.87 ^a
	PM3	80.9279 ± 0.58 ^a	79.2514 ± 0.62 ^a
Tumpat	T1	80.9639 ± 0.75 ^a	78.4476 ± 1.19 ^a
	T2	81.0533 ± 0.80 ^a	78.0449 ± 0.40 ^a
	T3	80.9462 ± 0.61 ^a	77.9933 ± 1.86 ^a

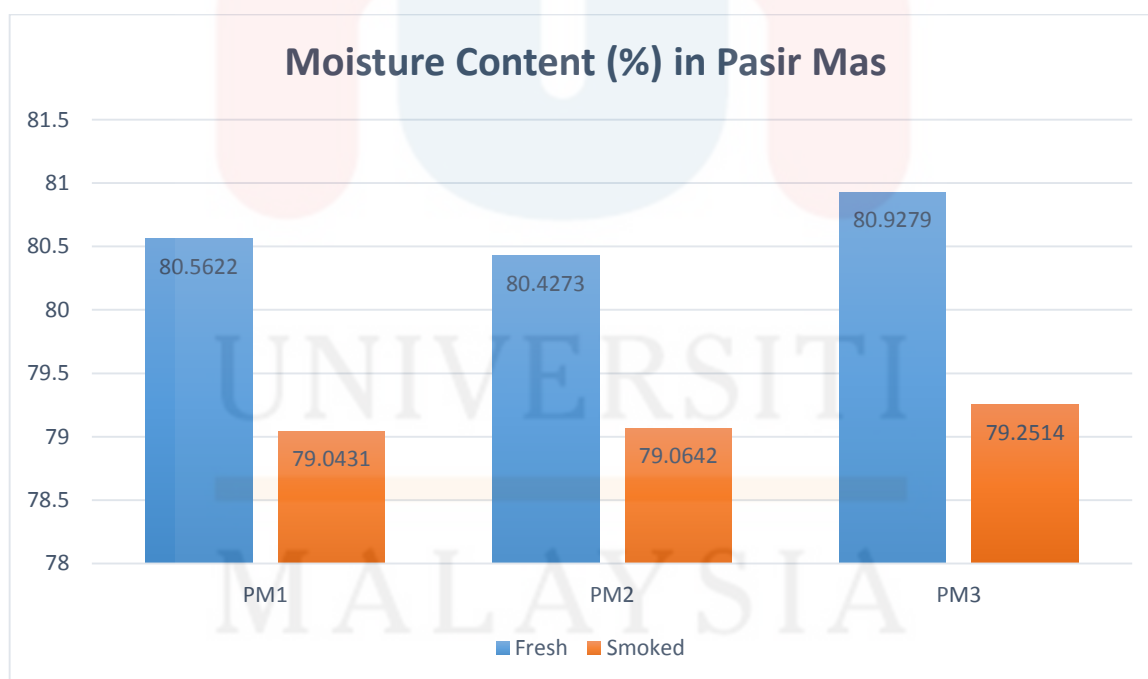


Figure 4.1 : Moisture content (%) of *C. fluminea* from Pasir Mas in different treatment.

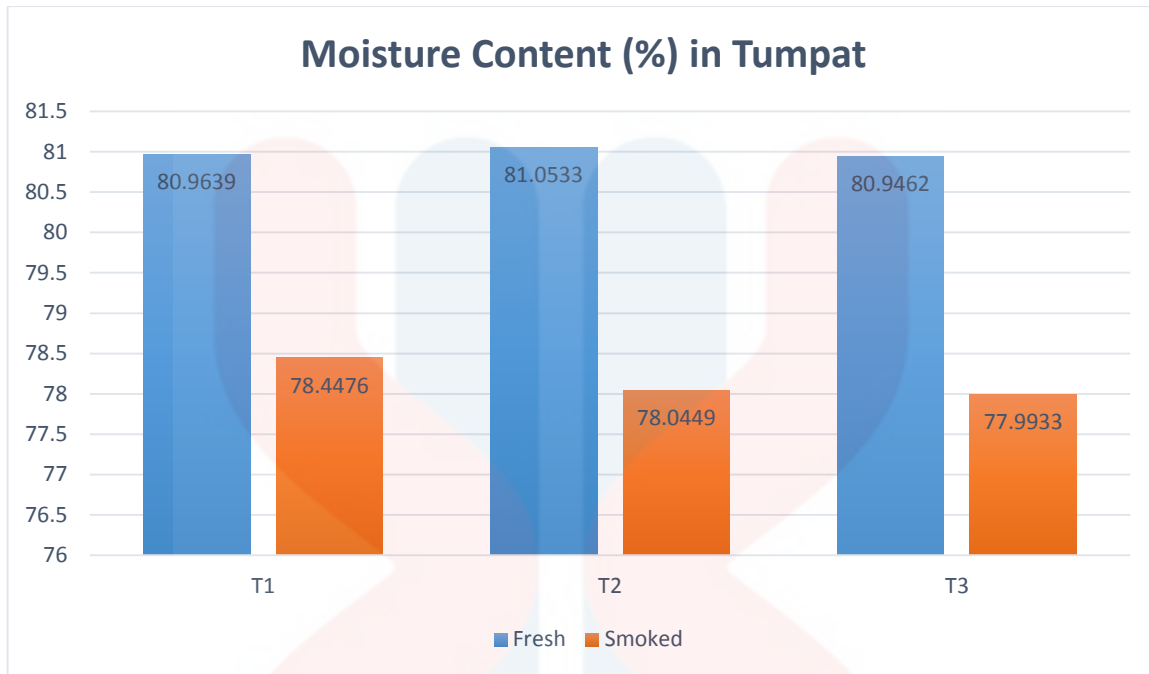


Figure 4.2 : Moisture content (%) of *C. fluminea* from Tumpat in different treatment.

As we can see from the Figure 4.1 and Figure 4.2, moisture content of fresh sample is higher compare to smoked sample. Among the two harvested location, the highest moisture content is T2 (81.0533 ± 0.80^a) while the lowest moisture content is PM2 (80.4273 ± 0.58^a). However, according to Table 4.1, the moisture content of sample from both harvested location and different treatment was not significantly different ($p > 0.05$).

The value of moisture content in smoked sample is lower compare to fresh sample because of the loss in moisture during hot smoking. Smoking process is one of the preservation method that can lowering the water activity and the water activity can effect the moisture content. “Water activity is a central factor that affects food composition, stability, safety and nutritive appeal”. Smoked sample has the lowest value of moisture content because it has lower water activity compare to fresh sample.

However, from Figure 4.3 and Figure 4.4, there are slightly different between the values of moisture content of smoked sample from both harvested location. The value of moisture content in smoked sample from Pasir Mas is higher compare to the value of moisture content in fresh sample from Tumpat. This is due to the ingredients use in marinating the *Corbicula* before smoking process.

The ingredients for marinating the smoked *Corbicula* also effect the value of moisture content. The ingredients for marinating the *Corbicula* are garlic, onion, lemon grass, sugar, salt and seasoning powder. According to “Preservation methods that involve lowering the water activity of foods are addition of salt, addition of sugars or sugar alcohols, drying, freeze drying and freezing”. The higher the temperature of the smoking process, the lower the value of the moisture content. According to Bouriga, Ismail, Gammoudi, Faure, & Trabelsi, (2012) Moisture content decreased with increasing temperature.

4.1.2 Ash Content

Table 4.2 shows the relationship between the ash content of *Corbicula fluminea* in two different harvested location which are from Pasir Mas and Tumpat and the ash content of *Corbicula fluminea* from different treatment which are fresh and smoked.

Table 4.2 : Ash content (%) \pm S.D for different treatment.

Harvested location / Fresh		Ash Content (%) \pm S.D.	
		Fresh	Smoked
Pasir Mas	PM1	0.7940 \pm 0.38 ^a	1.2918 \pm 0.53 ^a
	PM2	0.8260 \pm 0.30 ^a	1.3974 \pm 1.91 ^a
	PM3	0.8728 \pm 0.23 ^a	1.3913 \pm 0.61 ^a
Tumpat	T1	0.7824 \pm 0.40 ^a	1.1927 \pm 0.82 ^a
	T2	0.5478 \pm 0.07 ^a	0.8233 \pm 0.77 ^a
	T3	0.8207 \pm 0.42 ^a	1.1047 \pm 0.65 ^a

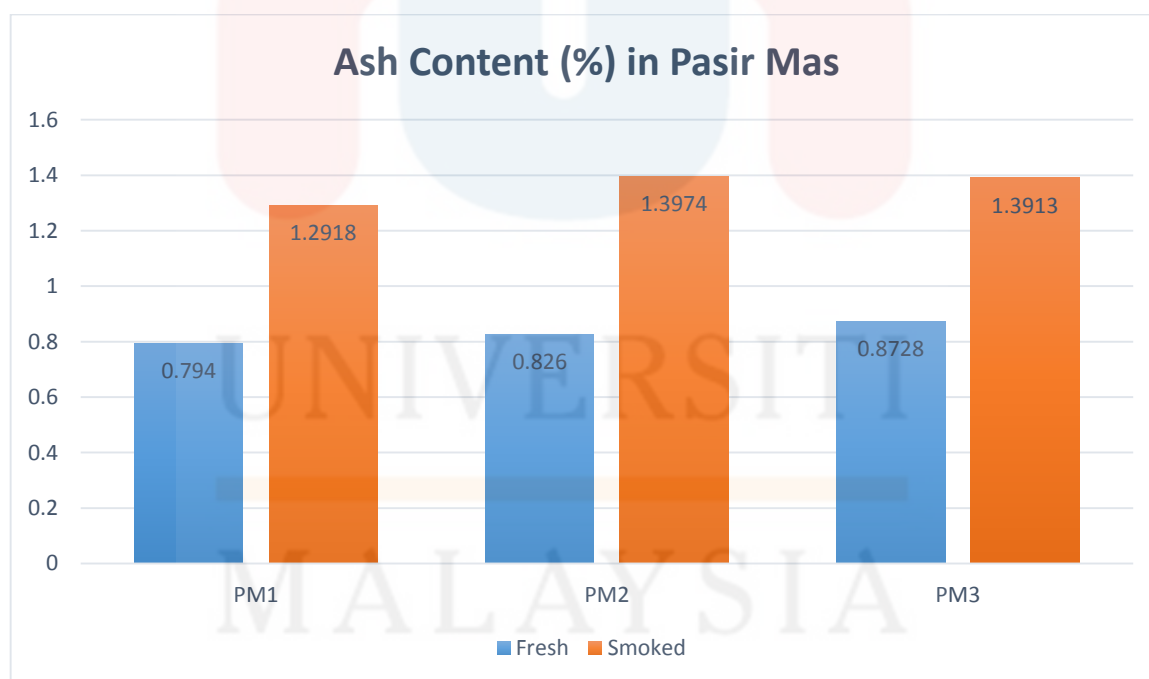


Figure 4.3 : Ash content (%) of *C. fluminea* from Pasir Mas in different treatment.

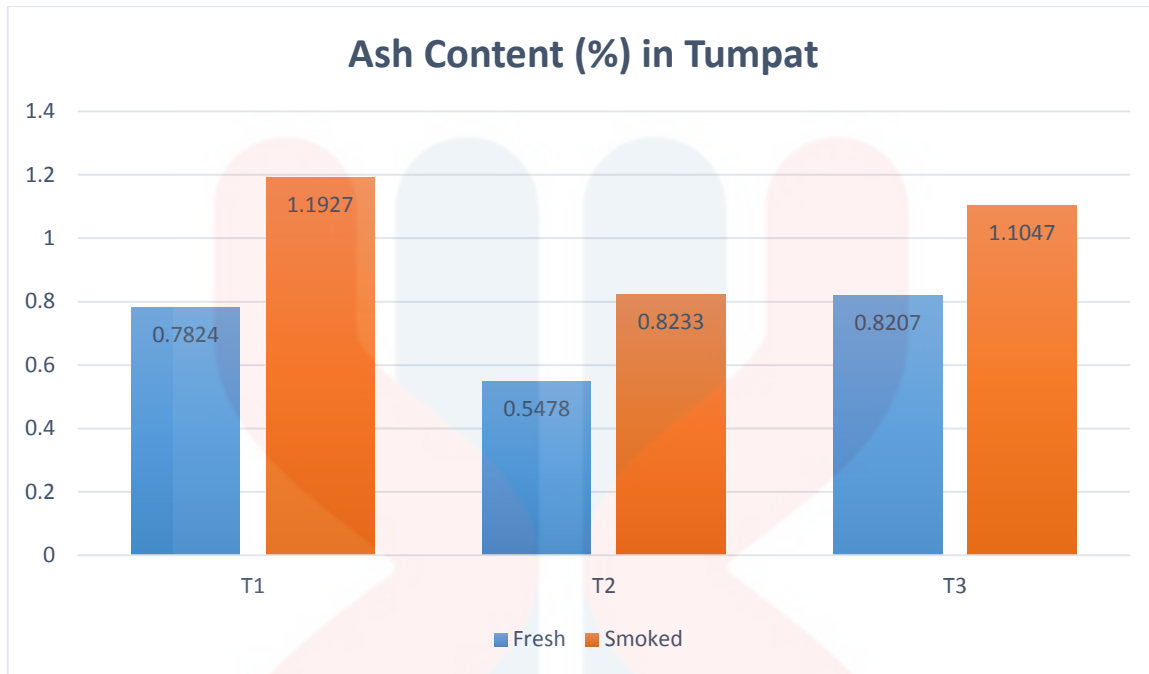


Figure 4.4 : Ash content (%) of *C. fluminea* from Tumpang in different treatment.

As we can see from the Figure 4.3 and Figure 4.4, ash content of smoked sample is higher compare to fresh sample. Among the two harvested location, the highest ash content is PM2 (1.3974 ± 1.91^a) while the lowest moisture content is T2 (0.5478 ± 0.07^a). However, according to Table 4.2, the ash content of sample from both harvested location and different treatment was not significantly different ($p > 0.05$).

From table 4.1, the moisture content of fresh sample is higher compare to smoked sample. The lower the value of moisture content, the higher the value of ash content. This is due to the factor that effect the value of ash which is temperature. The temperature that use in determination ash is 440°C for 24 hours. The higher the temperature use, the higher the value of ash. As we know that, smoking process loss the moisture of the sample. According to (Bouriga et al., 2012) ash content increased with increasing temperature and time.

However, from Figure 4.3 and Figure 4.4, there are slightly different between the values of ash content of smoked *Corbicula* from both harvested location. The value of ash content in smoked sample from Pasir Mas is higher compare to the value of ash content in fresh sample from Tumpat. This is because, different location use different temperature to smoked the *Corbicula*. Other than that, in Pasir Mas, the quantity of *Corbicula* that seller smoked every day is lower compare to in Tumpat. The less quantity of *Corbicula* that the seller smoked, the easier the *Corbicula* to be smoked. Furthermore, different seller use different type of smoking process.

4.1.3 Crude Fat Content

Table 4.3 shows the relationship between the crude fat content of *Corbicula fluminea* in two different harvested location which are from Pasir Mas and Tumpat and the crude fat content of *Corbicula fluminea* from different treatment which are fresh and smoked.

Table 4.3 : Crude fat content (%) \pm S.D for different treatment.

Harvested location / Fresh		Crude Fat Content (%) \pm S.D.	
		Fresh	Smoked
Pasir Mas	PM1	11.6362 \pm 1.1 ^a	9.9461 \pm 2.25 ^a
	PM2	11.6367 \pm 0.49 ^a	10.3285 \pm 1.26 ^a
	PM3	11.0669 \pm 1.23 ^a	9.6044 \pm 1.22 ^a
Tumpat	T1	9.9006 \pm 0.87 ^a	10.7055 \pm 0.45 ^a
	T2	9.2637 \pm 2.12 ^a	10.9262 \pm 1.65 ^a
	T3	9.8788 \pm 1.31 ^a	10.9277 \pm 0.74 ^a

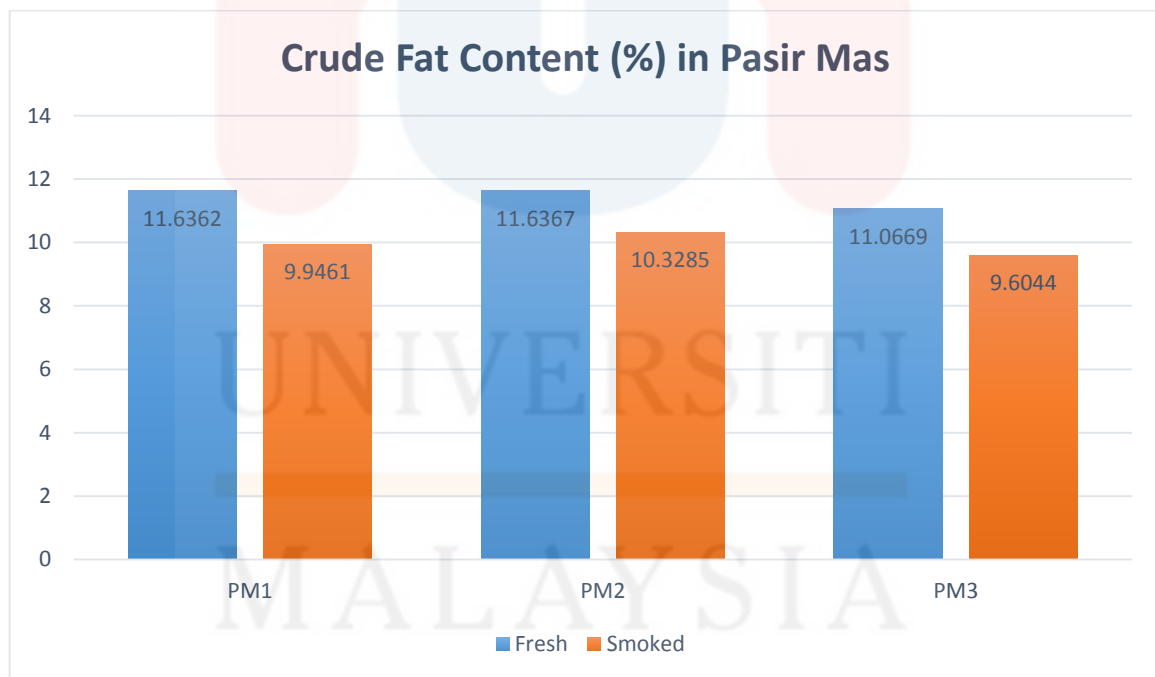


Figure 4.5 : Crude fat content (%) of *C. fluminea* from Pasir Mas in different treatment.

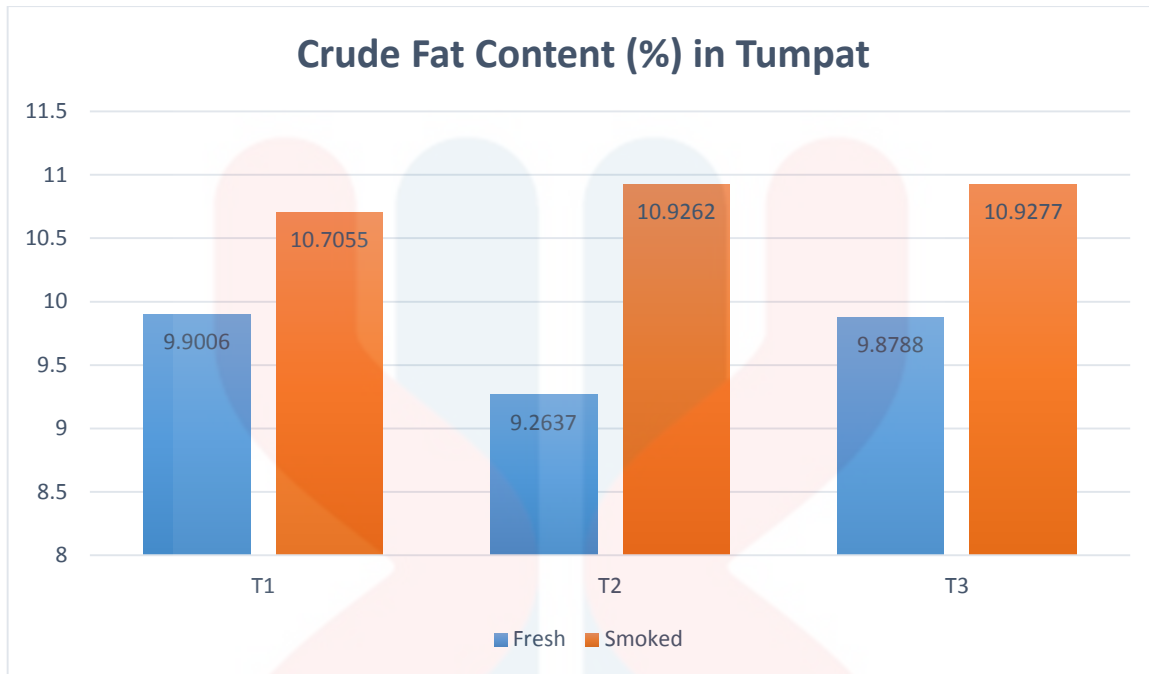


Figure 4.6 : Crude fat content (%) of *C. fluminea* from Tumpat in different treatment.

As we can see from the Figure 4.5 and Figure 4.6, crude fat content of fresh sample is higher compare to smoked sample in Pasir Mas while crude fat of smoked sample is higher compare to fresh sample in Tumpat. Among the two harvested location, the highest crude fat content is fresh PM2 (11.6367 ± 0.49^a) while the lowest moisture content is fresh T2 which is (9.2637 ± 2.12^a). However, according to Table 4.1, the crude fat content of sample from both harvested location and different treatment was not significantly different ($p > 0.05$).

Based on Figure 4.5, the value of crude fat content in fresh sample is higher compared to smoke sample and based on Figure 4.6, the value of crude fat content in smoked sample is higher compare to fresh sample. However, according to Aba, (2013), crude protein, crude lipid, crude fibre, and ash content increased with increasing temperature and time. The crude fat content supposedly higher in smoked sample but in Pasir Mas, shows that fresh sample contain high crude fat because heat processing methods increases the value of crude fat.

However, the reduction in crude lipid could be attributed to possible loss of fat due to the high temperature as observed in earlier studies (Ali, Ahmadou, Mohamadou, Saidou, & Tenin, 2011).

4.1.4 Crude Protein Content

Table 4.4 shows the relationship between the crude protein content of *Corbicula fluminea* in two different harvested location which are from Pasir Mas and Tumpat and the crude protein content of *Corbicula fluminea* from different treatment which are fresh and smoked.

Table 4.4 : Crude protein content (%) \pm S.D for different treatment.

Harvested location / Fresh		Crude Protein Content (%) \pm S.D.	
		Fresh	Smoked
Pasir Mas	PM1	6.2112 \pm 0.32 ^b	8.4282 \pm 0.50 ^b
	PM2	6.8613 \pm 0.50 ^{ab}	8.5790 \pm 0.40 ^{ab}
	PM3	6.3780 \pm 0.39 ^{ab}	9.2074 \pm 1.54 ^{ab}
Tumpat	T1	7.8624 \pm 1.08 ^{ab}	9.1724 \pm 1.04 ^{ab}
	T2	8.1898 \pm 0.89 ^a	9.1143 \pm 1.15 ^a
	T3	7.5931 \pm 1.30 ^a	9.6575 \pm 1.15 ^a

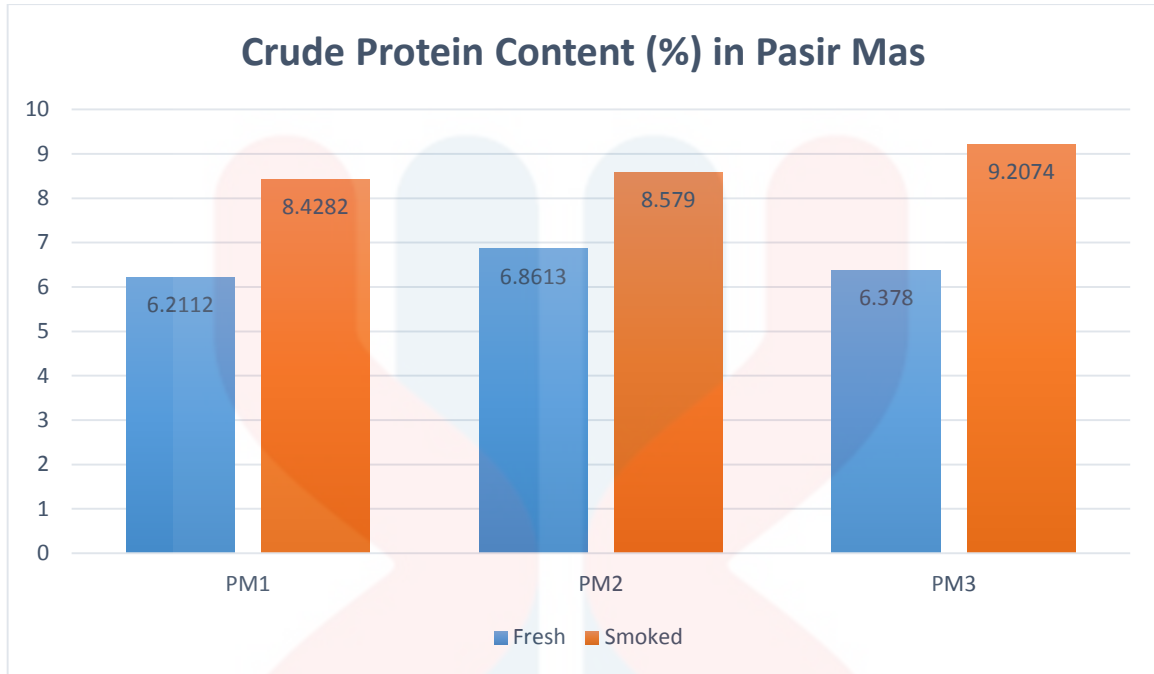


Figure 4.7 : Crude protein content (%) of *C. fluminea* from Pasir Mas in different treatment.

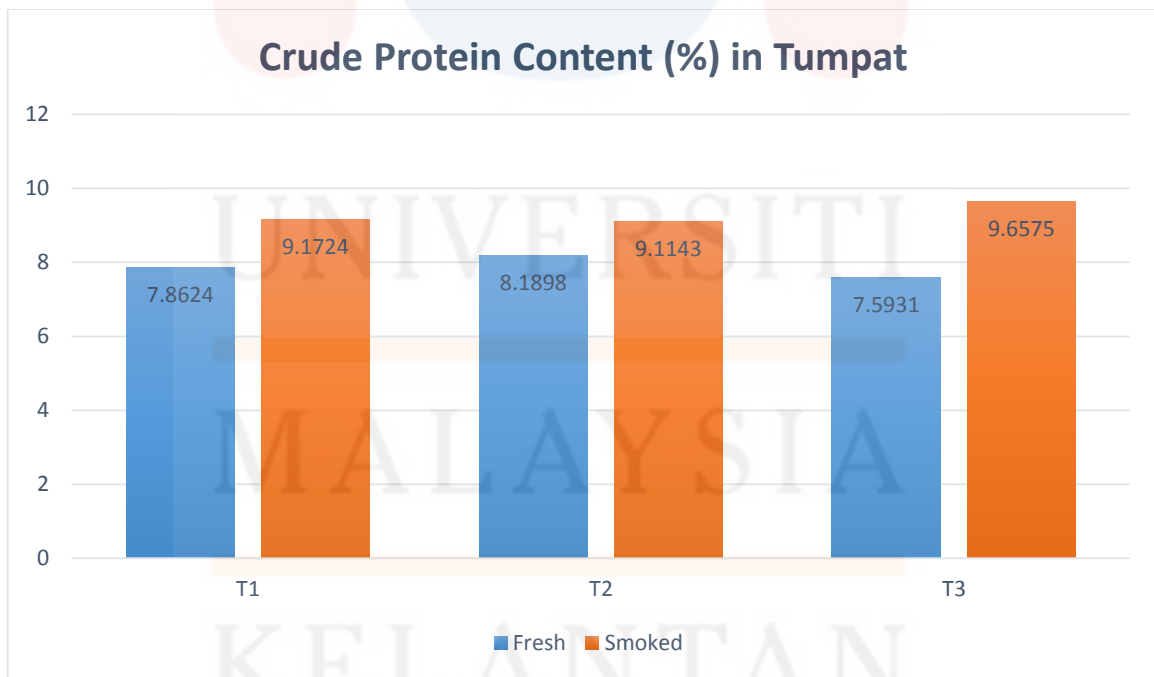


Figure 4.8 : Crude protein content (%) of *C. fluminea* from Tumpat in different treatment.

As we can see from the Figure 4.7 and Figure 4.8, crude protein content of smoked sample is higher compare to fresh sample in both location. Among the two harvested location, the highest crude protein content for smoked sample is T3 (9.6575 ± 1.15^a) while the lowest crude protein content is smoked is PM1 (8.4282 ± 0.50^b). Other than that, the highest crude protein content for fresh sample is T2 (8.1898 ± 0.89^a) while the lowest crude protein content is PM1 (6.2112 ± 0.32^b). However, according to Table 4.1, the crude fat content of sample from both harvested location and different treatment was not significantly different ($p>0.05$).

From Table 4.1, shows that, smoked Corbicula has lower moisture content due to the loss of water in smoking process. In this research, the crude protein content of smoked sample has the higher value compare to the fresh sample. According to Aliya Al Ghabshi, Humaid Al-Khadhuri, Nasser Al-Aboudi, Sami Al-Gharabi, Aziz Al-Khatiri, (n.d.) protein levels increased with decreasing moisture content and according to Akintola, (2015) Proximate analyses showed that on dry weight basis smoking increased the protein and carbohydrate values

4.1.5 Carbohydrate Content

Table 4.5 shows the relationship between the carbohydrate content of Corbicula fluminea in two different harvested location which are from Pasir Mas and Tumpat and the carbohydrate content of Corbicula fluminea from different treatment which are fresh and smoked.

Table 4.5 : Carbohydrate content (%) \pm S.D for different treatment.

Harvested location / Fresh		Carbohydrate Content (%) \pm S.D.	
		Fresh	Smoked
Pasir Mas	PM1	1.3708 \pm 0.49 ^a	0.8911 \pm 0.90 ^a
	PM2	0.8062 \pm 0.43 ^a	0.5481 \pm 0.17 ^a
	PM3	0.9740 \pm 0.84 ^a	0.9728 \pm 0.90 ^a
Tumpat	T1	0.5882 \pm 0.63 ^a	0.6695 \pm 0.35 ^a
	T2	0.8918 \pm 0.90 ^a	0.9262 \pm 0.85 ^a
	T3	0.6878 \pm 0.78 ^a	0.3234 \pm 0.44 ^a

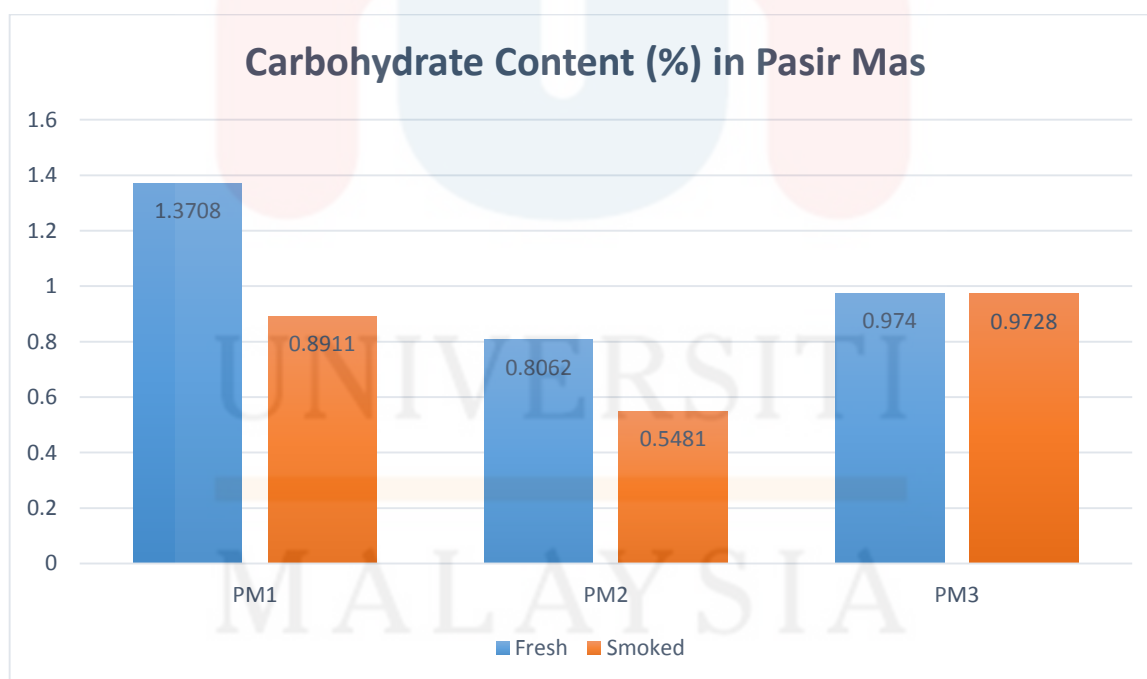


Figure 4.9 : Carbohydrate content (%) of *C. fluminea* from Pasir Mas in different treatment.

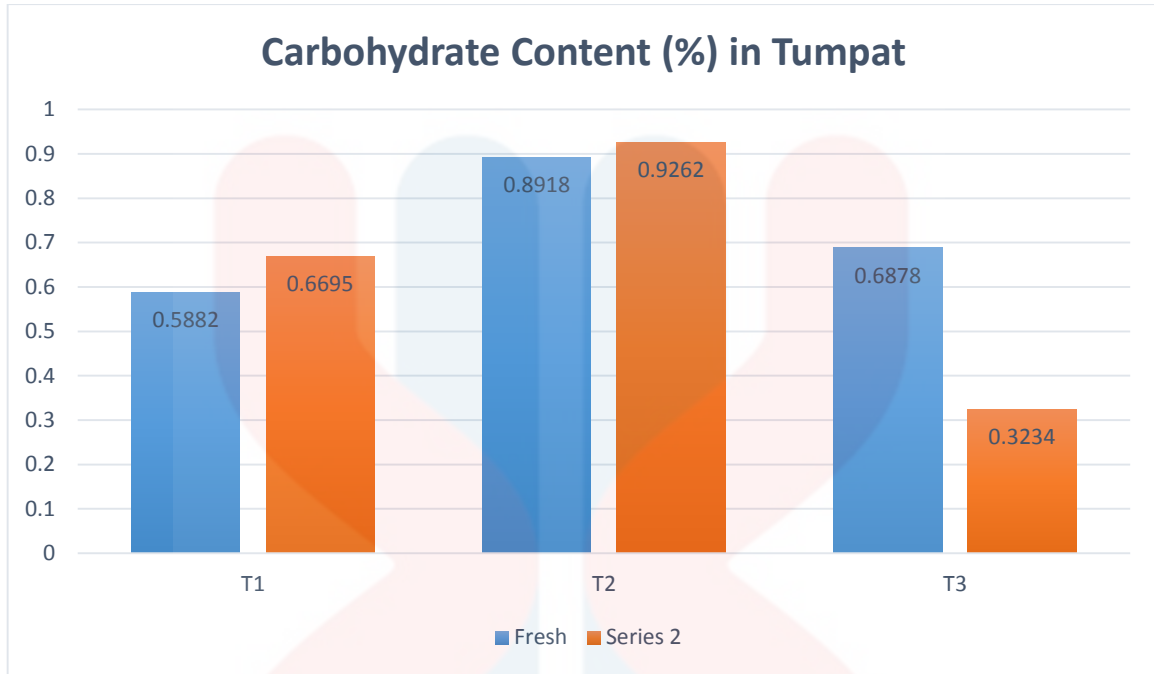


Figure 4.10 : Carbohydrate content (%) of *C. fluminea* from Tumpat in different treatment

As we can see from the Figure 4.9 and Figure 4.10, carbohydrate content of smoked from both location are not accurate. For carbohydrate content, this research use method can lead to erroneous results due to experimental errors in any of the other methods. Among the two harvested location, the highest carbohydrate content is Fresh PM1 (1.3708 ± 0.49^a) while the lowest carbohydrate content is smoked T3 (0.3234 ± 0.44^a). However, according to Table 4.5, the crude fat content of sample from both harvested location and different treatment was not significantly different ($p > 0.05$).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In the present study, nutritional value of *Corbicula fluminea* has been observed. For moisture content, obviously fresh *Corbicula* has the highest value (81.0533 ± 0.80^a) compare to smoked *Corbicula*. Next, for ash content, smoked *Corbicula* from Tumpat has the highest value (1.3974 ± 1.91^a) while fresh *Corbicula* from Tumpat has the lowest value (0.8233 ± 0.77^a). Order than that, for crude fat, fresh *Corbicula* from Pasir Mas has the highest value (11.6362 ± 1.1^a) while the lowest value ($9.2637 \pm 2.12a$) is fresh *Corbicula* from Tumpat. For crude protein, the highest value ($9.6575 \pm 1.15a$) is smoked *Corbicula* from Tumpat while fresh *Corbicula* from Pasir Mas has the lowest value ($6.2112 \pm 0.32b$). Last but not least, for carbohydrate, fresh *Corbicula* from Pasir Mas has the highest value (1.3708 ± 0.49^a) while smoked *Corbicula* from Tumpat has the lowest value (0.3234 ± 0.44^a). It can be concluded that, the cooking method such as smoked can increase the the nutritional value of *Corbicula fluminea*.

5.2 Recommendation

Future work on investigation of nutritional composition shall be conducted on *Corbicula fluminea*, Asian clams. Moreover, there is great to know the exact nutritional value because it can be benefits to the consumers. Thus, suitable methods for determining the exact nutritional value should be used. In the other hand, since we are lacking the information about *Corbicula fluminea* , future work on the chemical and physical properties also can be done. Some physical properties such as pH value and water activity of the *Corbicula* can be benefits to other people.

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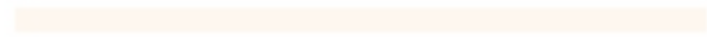
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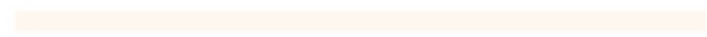
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APPENDICES



Seller from Pasir Mas



Seller from Tumpat



Extraction of fat by Soxhlet



Distillation and titration process in determination of protein

MALAYSIA
KELANTAN

MOISTURE

Duncan^{a,b}

LOCATION	N	Subset
		1
T3	6	79.469733
T2	6	79.549100
T1	6	79.705783
PM3	6	79.745733
PM1	6	79.802650
PM2	6	80.089650
Sig.		.297

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

b. Alpha = .05.

ASH

Duncan^{a,b}

LOCATION	N	Subset
		1
T2	6	.685567
T3	6	.962667
T1	6	.987517
PM1	6	1.042917
PM3	6	1.111750
PM2	6	1.132083
Sig.		.148

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

b. Alpha = .05.

FAT

Duncan^{a,b}

LOCATION	N	Subset
		1
T2	6	10.094967
T1	6	10.303067
PM2	6	10.335638
T3	6	10.403217
PM1	6	10.791133
PM3	6	10.982583
Sig.		.357

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

b. Alpha = .05.

PROTEIN

Duncan^{a,b}

LOCATION	N	Subset	
		1	2
PM1	6	7.354667	
PM3	6	7.720150	7.720150
PM2	6	7.792717	7.792717
T1	6	8.517400	8.517400
T3	6		8.620300
T2	6		8.652083
Sig.		.053	.128

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

b. Alpha = .05.

CARBO

Duncan^{a,b}

LOCATION	N	Subset
		1
T3	6	.505633
T1	6	.628833
PM3	6	.677133
T2	6	.908967
PM2	6	.973417
PM1	6	1.130983
Sig.		.142

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

b. Alpha = .05.