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### Full Length Research Article

## QUALITY ANALYSIS OF THE INDIAN SCAD OIL (*DECAPTERUS SP*) AS A FUNCTIONAL MICROENCAPSULE IN THE BROWNIES

<sup>1</sup>Luthfiah, <sup>2</sup>Jalil Genisa, <sup>3</sup>Effendi Abustam and <sup>4</sup>Metusalach

<sup>1</sup>Doctoral Program, Postgraduate of Hassanuddin University

<sup>2</sup>Department of Food Technology-Faculty of Agriculture-Unhas-Makassar

<sup>3</sup>Department of Livestock Products Technology-Faculty of Animal Sciences Unhas-Makassar

<sup>4</sup>Department of Fisheries Resource Utilization-Faculty of Fisheries-Unhas-Makassar

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#### ABSTRACT

The study aims to describe the differences in quality, yield and composition of  $\omega$ -3 fatty acids in fish oil, and quality microencapsule as functional materials on brownies. The research was conducted in three stages, namely extraction, testing the quality, yield and composition of  $\omega$ -3 fatty acids and proximate of brownies. Analysis of quality consist of the composition of  $\omega$ -3 fatty acids, yield, peroxide, anisidin, and total oxidation. Proximate data were analyzed with ANOVA and continued with HSD at level  $\alpha$  of 5%. The results showed that yield of the method BD is 4.05% and SP is 3.70%. Quality parameters namely peroxide, anisidin and total oxidation included in IFOMA standard.  $\Omega$ -3 fatty acids EPA 26.9 mg/g and DHA 16.10 mg/g for the method BD, while the EPA from methods SP was 4.77 mg/g and DHA 15.29 mg/g. The microencapsule yield ranged from 46.86 to 68.90%. The composition of  $\omega$ -3 EPA of layang fish oil concentrate in the microcapsules increased and DHA also increased. The results of the scoring analysis of the parameters proximate with the addition of 10% microencapsule and cold storage at 5 °C gives the best result on the first day than other treatments.

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#### INTRODUCTION

Nowadays fatty of the fish, especially fish of certain species get a lot of attention from food experts and health because on the oil fish found group of unsaturated fatty acids omega-3 (n3), namely eicosapentaenoic acid (20: 5, n-3) and fatty acid docosahexaenoic (22: 6 n-3). Unsaturated fatty acids (PUFA) are configured omega-3 that plays a role in disease prevention arteriosclerosis (clogged arteries) and coronary heart disease (Yunika, 1995). Omega-3 fatty acids have a special meaning in nutrition because they contain fatty acids that are related to health and intelligence. Fatty acids are related to health are EPA (Eicosapentaenoic Acid). While fatty acids are associated with intelligence is DHA (Docosahexaenoic Acid) (Nettleton in Mercy, 2006). DeKelbaum and Akabas (2006) explains that EPA and DHA can reduce the accumulation of cholesterol in the arterial wall. Based Omega-3 benefits to health, people who eat at least 50 grams of fish per week can reduced 50% probability heart disease.

EPA stimulate the body to produce prostacyclin, regulate blood pressure, lowering cholesterol and blood triglyceride, and reduce the pain of angina (heart pain) when the body motion (Simorangkir and Simorangkir, 1993). Whereas DHA increase intelligence of human. Sunarya (1993) suggested that DHA found in fish also contains vitamin A is beneficial in preventing night blindness and blindness. Fish oil is often applied in the food industry. However, fish oil is more sensitive than other oils. Unsaturated fatty acid levels are high, including EPA and DHA is very easily oxidized. In addition, the concentration of phospholipids and unsaturated fatty acids are high making fish oil is more sensitive than other oils. Therefore, the quality of oil in the food industry should receive serious attention. In the food industries, processing process affects the formulation of fish oil. To maintain the quality of the oil fish on the heating stage, the fish oil should be in a protected state in the form of microencapsule or emulsion. Heating may cause oxidation run faster, and first compound that undergoes oxidation is unsaturated fatty acids (PUFA) such as omega-3 fatty acids. Omega-3 fatty acids derived from plant foods, such as soy, corn and olives. Beside plant food, omega-3 fatty acids are also derived from fishery products.

\*Corresponding author: Luthfiah

Doctoral Program, postgraduate of Hassanuddin University

Excess fishery products is quite high protein content (20%), fish meat is easily digested, contain unsaturated fatty acids with very low cholesterol levels, and contains minerals (K, Cl, P, S, Mg, Ca, Fe, Zn, F, Ar, Cu) as well as vitamins A and D. One of fishery products which have a high nutritional value, the layang fish. Layang Fish (*Decapterus sp*) is one of communities of the small pelagic are relatively abundant in the waters of Indonesia so that potential as a source of fish oil to support the food industry, such as brownies. Brownies is a cake that very liked by community and marketing cycle already reach all people, in addition, it's easy to make in home industry. According Ningrum (2012), the brownies are a typical American cake that was first recognized in 1987. Brownies classified as type of cake that has a high glycemic index means by consuming brownies, blood sugar can quickly rise so soon after eating brownies will be more fresh bodies.

Brownies also contain complete vitamins such as vitamin C, thiamin, riboflavin, niacin, pantothenic acid, vitamin B6 and vitamin B12. Brownies can be categorized into two, namely baked brownies and steamed brownies. Based on the research Saragih in Ningrum (2012), generally there is no difference between them. The difference lies in the content of water in brownies. The steamed brownies have a higher water content than the baked brownies so it has a lower shelf. Base of the taste, the baked brownies more savory than the steamed brownies. However, in terms of health, steamed brownies are safer because they do not form free radicals as a result of the roasting process. To improve the nutritional value of brownies then given fish oil in the form of microcapsules. The addition of  $\omega$ -3 into the brownies will yield the brownies that rich  $\omega$ -3.

Until now, research the addition of omega-3 fish oil to formulate a stable microencapsules in the brownies had never been done. Based on this fact, it is very important study was conducted to determine the quality of fish oil, fatty acid composition of concentrate  $\omega$ -3 fatty acid composition and stability in brownies during cold storage. The urgency of this study is the reached information about the differences quality and composition of fatty acids of fish oil, concentrates and microencapsules on method of fish oil processing (Bligh and dyer) and (steam and press). Another urgency expected from this research that is the best formulation of fish oil concentrate ratio between omega-3 and coating (acacia gum and gelatin). In addition, the expected discovery microencapsules percentage comparisons were added in dough brownies to the fatty acid composition and proximate of the brownies quality and influence of cold storage 5 °C to quality brownies.

## MATERIALS AND METHODS

### Materials and equipment

Materials used in this study, namely fish oil from Layang fish (*Decapterus sp*), bentonite, NaOH, EDTA aqueous, alcohol, ethanol, HCl, n-hexane, hot urea, methanol, HCl, octyl gallate, acacia gum and gelatin, NaOH 0.5 N, NaCl, methanol (CH<sub>3</sub>OH), Isoctane, BF<sub>3</sub> 20%, Isooctane, anhydrous Na<sub>2</sub>SO<sub>4</sub>, Petroleum benzene (40-60 °C), distilled water, anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), neutral alcohol KOH, pp indicator, HCl, acetic acid, chloroform, hexane, oxalic acid, KI, starch, p-anisidin, hydrogen gas. The tools used in this study is a meat

grinder (Panasonic), glass tools. Centrifuge, a homogenizer, a spray dryer (Buchi 190 mini spray dryer), vortex (Hettich EBA-8), a hot plate, freezer, scales (qualtro), funnel and filter paper. Distillation equipment, evaporator (Eyela N 1000), a unit of gas chromatography (GCMS-QP2010), mixer (philips), gas stove, tube sokhet, distillation flask, burette, fat pumpkin, desiccator and spectrophotometers.

### Research Design

This study used a completely randomized design factorial. Making microencapsule consists of:

A1 = (55% gelatin: 10% gum acacia): 35% fish oil  
 A2 = (65% gelatin: 10% gum acacia): 25% fish oil  
 A3 = (70% gelatin: 10% gum acacia): 20% fish oil  
 Each consists of three replications.

Making brownies with the addition of  $\omega$ -3 mikroenkapsul consists of two factors, namely A (ratio of  $\omega$ -3 mikroenkapsul additions) which consists of three levels, namely A1: 10%; A2: 20%; and A3: 30%. Factor B (long storage at 5 °C), namely B1: the first day (H1); B2: third day (H3) and B3: sixth day (H6).

### Research Prosedure

This research was conducted in three phases of activity. The stages are as follows:

#### The first stage

Processing of Fish Oil. Fish oil processing performed by the method of Bligh and Dyer (1962). Further extraction of fish oil with steam and press method. Steamed fish with steam for 15 minutes at 90 °C. Fish that have been cooked wrapped in a cloth for pressing, and then to separate the liquid fraction (containing fish oil) and fish meal. Coarse fish oil is heated at a temperature of 60 °C. Bleaching performed at 60 °C, Sentrifuge at 60 rpm for 60 minutes with the addition of bentonite 2.5% (w/v) were activated in the muffle temperature of 300 °C for 3 hours. Furthermore filtered with filter paper to separate the dirt and adsorbent residue then produced fish oil so clear. Isolation of  $\omega$ -3 fatty acids by Medina *et al*, (1995). Making concentrate  $\omega$ -3 fatty acid by saponification and fractionation with urea. Furthermore, the transesterification by Anwar in Nanlohy (2008) as an initial treatment for fatty acid analysis by CG. Concentrated fish oil that has been tested to brownies. To ensure food safety, the total of microbial tested by Total Plate Count method (TPC) based Fardias, (1984). Further microencapsules manufacture. Gelatin and acacia gum dissolved in hot water (100 °C) in order to obtain a concentration of 10% (w/v). After cooled, oil rich in omega-3 fatty acids are mixed in order to obtain the ratio of the coating: stuffing materials 2: 1, 3: 1 and 4: 1. The addition of gelatin and gum acacia with composition ratio (gelatin: gum acacia) and fish oil as follows:

A1 = (55% gelatin: 10% gum acacia): 35% fish oil  
 A2 = (65% gelatin: 10% gum acacia): 25% fish oil  
 A3 = (70% gelatin: 10% gum acacia): 20% fish oil

Then homogenized at 2000 rpm for 15 minutes. The emulsion is dried with a spray dryer at an inlet temperature of 130 °C and outlet temperature of 72 °C with a flow rate of 5 mL / sec (Estiasih, 2008).

### The Second Stage

The second phase is done to test the quality, yield and composition of omega-3 fatty acids on mikroenkapsul that have been made previously. Tests carried out by Gas Chromatography (GC) to determine the content of  $\omega$ -3 fatty acids; and the yield based Sahena *et al.*, (2010); peroxide according to AOAC (2005); determining the value of Anisidin/p-AV base on Watson, (1994) and the total number of oxidation by Perrin, (1996). The parameters analyzed referring to IFOMA (International Fishmeal and Oil Manufacturers Association) of standards consumption of fish oil.

### The Third Stage

**(1) Making brownies and Additions Microencapsule:** A total of 30% of eggs and 20% sugar, whipped until fluffy, then add 2% TBM, 1% vanilla and 7% milk powder. Once homogeneous, add flour 20% and 10% chocolate. Then added 10% vegetable oil. Steaming batter performed for 20 minutes at a temperature of 95 °C. The temperature is kept constant. Furthermore added microencapsule concentrate omega-3, respectively 10%, 20% and 30%. Storage at 5 °C. Furthermore, analyzed of the proximate, total microbial, respectively on the first day, third and sixth.

**(2) Proximate Analysis:** Parameters to be observed is moisture content, ash content, protein content, fat and carbohydrate content analysis according to AOAC (2005).

### Data Analysis Techniques

Data extraction of omega-3, the total microbial composition of omega-3 fatty acids, peroxides, value Anisidin/p-AV and total oxidation analyzed descriptively and displayed in table form. Data of proximate were analyzed with ANOVA and continued with honest significant difference test at level  $\alpha$  of 5% (Steel, RGD and JH Torrie. 1989; Sugiyono, 2013; Siregar, 2013).

## RESULTS AND DISCUSSION

### A. Yield and Quality of Layang Fish Oil

The results of the analysis of the yield and quality of fish oil with the method (steam and press) and method (Bligh and dyer) shown in Table 1.

Table 1. Yield and Quality of Layang Fish Oil

No	Parameters	Blig and Dyer Method	Steam and Press Method	Quality Standards of IFOMA
1	Yield	4,05%	3,07%	-
2	Peroxide	8,50 meq/kg	8,20 meq/kg	3-20 meq/kg
3	Anisidin	9,34	8,56	4-60
	Total oxidation	26,34	24,96	10-60

Source : Primery Data from Integrated Laboratory IPB, 2014

Based on Table 1, it can be argued that the value of the yield was below 5%. Peroxide value, anisidin and total oxidation of two standard methods of extraction are in IFOMA. Based on the quality parameters of fish oil are processed with the method (Bligh and Dyer) and methods (steam and press) it was concluded that fish oil overpass meet quality standards of IFOMA. Rich oil quality parameters  $\omega$ -3 fatty acids based on the level of oxidation that peroxide, anisidin, and total oxidation in which these three parameters have met quality standards IFOMA. Estiatih T (2008) and Dugan Jr., (1996), argued that the peroxide showed a new level of oxidation that occurs as the primary oxidation products. Anisidin Numbers indicate the level of oxidation that has past the secondary oxidation products of degradation of primary oxidation products (IUPAC, 1979). Further explained that the total number of oxidation is an indicator of the level of total oxidation of both recent and past that. It can be concluded that the rich oil  $\omega$ -3 fatty acids from fish fresh kite suitable for consumption by the quality standards. The yield of the extraction method (Bligh and Dyer) is 4.05% and the yield for the method of steam and Press is 3.70%.

### B. Fatty Acid Profile Of Fresh Layang Fish

Results of analysis of fatty acid composition of fresh fish by GC-MS showed that the extraction of fresh flying fish oil with steam and press method detected 77 peaks and identified 25 peak, while the method of Bligh and dyer detected 92 peaks and identified 22 peak. The composition of fatty acids were identified from both extraction methods are summarized in Table 2.

Table 2. Composition of fatty acid

No	Asam Lemak	Simbol	Metode Bligh dan Dyer	Metode Steam dan Press
saturated:				
1	caprylate	C18:0	nd	-
2	caproate	C10:0	nd	nd
3	lauric	C12:0	nd	0,06
4	Tridekanoat	C13:0	-	0,04
5	myristate	C14:0	0,28	3,24
6	Pentadekanoat	C15:0	0,03	0,75
7	palmitate	C16:0	3.14	16.97
Unsaturated:				
1	palmitoleic	C16:1	0,93	4,16
2	Cis-10-heptadekanoat	C17:1	0,10	0,22
3	elaidic	C18:1n9t	0,16	0,12
4	oleic	C18:1n9c	11,01	8,51
5	linoleic	C18:2n6c	1,06	0,96
6	$\gamma$ -linolenic	C18:3n6	0,20	0,10
7	Cis-11-Eicosanoat	C20:1	1,53	0,30
8	linolenic	C18:3n3	0,53	0,57
9	Cis-11,14-Eicosadienoat	C20:2	0,31	0,46
10	Cis-8,11,14-Eicosatrienoat	C20:3n6	0,28	nd
11	Erucik	C22:1n9	-	0,07
12	arachidonic	C20:4n6	1,62	1,69
13	Cis-	C20:5n3	26,98	4,77
14	5,8,11,14,17Eicosapentaenoat	C24:1	0,65	0,40
15	Nervonik	C22:6n3	16,10	15,29
	Cis4,7,10,13,16,19Docosaheksanoat			

Source: Primery Data from Integrated Laboratory IPB, 2014

Based on Table 2, stated that the EPA value on the method (Bligh and Dyer) was 26.9 mg/g, while the method (Steam and

Press) was 4.77 mg/g. DHA values in method (Bligh and Dyer) of 16.10 mg/g and DHA on Steam and Press method was 15.29 mg/g.

### C. EPA And DHA Levels Before and After Crystallization

EPA and DHA can be assayed both before and after crystallization. In this study, levels of EPA and DHA enrichment is 2:04 times while the results of previous studies (Estiasih, *et al.*, 2008) showed that the level of enrichment based on the relative quantity (standard normalization method) is 1.81. The results of the analysis of the levels of EPA prior to crystallization was 4.86 mg/g, and after crystallization of 25.76 mg g, an increase of 5.3 times. While the levels of DHA before crystallization is 16:45 mg/g, and after crystallization of 17.67 mg/g, an increase of 1.07 times.

### D. Comparison Of Omega-3 (EPA And DHA) Without Oil In Dough Brownies With Addition Of Fish Oil

The results of the analysis of EPA and DHA in brownies with the addition of fish oil the highest concentration of 30% indicates that the brownies detected EPA and DHA, while the brownies without the addition of fish oil is not found omega-3 fatty acids. Extraction of oil from brownies found saturated fatty acids from vegetable oils namely caprylic, caproic, lauric, myristic, palmitic, stearic and heptadecanoat and unsaturated fatty acids from vegetable oils such as palmitoleic, oleic and linoleic.

### E. Microencapsule

Comparison of yield mikroenkapsul in each treatment are shown in Table 3.

**Table 3. Yield Mikroenkapsul Treatment Comparison Between Fish Oil and Coating Materials**

Treatment	Oil volume (ML)	GUM Acacia (ML)	GELATIN	Weight Of final capsule	Final oil (ML)	First oil (ML)	Yield (%)
A1	35	10	55	49.250	16.40	35	46.86
A2	25	10	65	53.800	13.45	25	53.80
A3	20	10	70	68.900	13.78	20	68.90

Source: Primary Data from Integrated Laboratory IPB, 2014

Based on Table 3, it was concluded that the yield on treatment A1, A2 and A3 respectively is 46.86%; 53.80% and 68.90%. The yield is measured as a percentage of the final weight divided by weight of petroleum oils beginning of microencapsule generated. The highest yield is on the A3 treatment applied to microencapsule brownies. Yield parameters were measured as variables that determine the economic feasibility of the production process microencapsule rich oil  $\omega$ -3 fatty acids. The ideal value of the yield is 100%, but in reality the yield obtained from a variety of coating materials stuffing ratio ranged from 46.86 to 68.90%. According Estiasi T., (2008), that on the microencapsule process can occur stuffing material loss during drying. Stuffing material on the surface of the globules oil in the emulsion leaving an atomizer or stuffing materials which migrate to the surface, leaving the surface of the particles prior to the formation of the hardened layer. Possibility of internal movement stuffing materials before the formation of the hardened layer.

### F. Test of Total Plates Count

Test of Total plate count is used to test the microbial contamination in a food product. The analysis showed that the value of the total number of all treatment ranges between 0 CFU -  $2,4 \times 10^3$  CFU. This value can be tolerated because it is still below the maximum value limit is  $1 \times 10^4$  colonies/gram (30 °C 72 hours) set by BSN (National Standardization Agency) with ISO: 7388 2009 The maximum limit of microbial contamination in food ICS 6.72102 million. The storage of special bakery products (sweet, salty, savory) at low temperature can prevent damage of product. Low temperatures can inactivate the enzyme so it can not decompose organic material contained in the brownies. Based on the Total Plate Count, it can be argued that the brownies were added microencapsule meet the safety requirements for consumption.

### G. Additional 10%, 20% and 30% Microencapsule on Brownies

Addition microencapsule on brownies with a concentration of 10%, 20% and 30% indicate that the higher the concentration microencapsule added to brownies, the higher the concentration of EPA and DHA. The analysis showed that the concentration of EPA and DHA in addition microencapsule concentration of 10%, 20% and 30% respectively were 12.15 mg/g; 13.86 mg/g; 18.82 mg/g, and 8:39 mg/g; 9.36 mg/g; and 13 mg/g.

### H. Effect of Long Storage Brownies With Addition Of 30% Microencapsule Toward Epa And Dha Content

The results of the analysis of the effect of storage time at a temperature of 5 °C in the first, the third and sixth day showed

that the longer the storage brownies, the content of EPA and DHA decreased. EPA decreased from 18.82 mg/g to 9:45 mg/g, whereas DHA reduced 13 mg/g to 6473 mg/g.

### Proximate Brownies

#### Moisture Brownies

Water is an important component of food which can manifest in various forms and different amounts. Water can be either intracellular or extracellular components of vegetable and animal materials. Water can function as a dispersing medium or solvent in various food products, as a dispersed phase in the emulsion products (butter and margarine), or as a minor component in the material/dry food products. Water in food plays a role in influencing the level of freshness, stability, durability, and ease of occurrence of chemical reactions, enzyme activity, and microbial growth (Kusnandar, 2010). The results of the analysis of water content brownies shows the average water content by the method of heat (Oven) ranged

from 28.18 to 32.78%. Lowest water levels obtained in 10% microcapsule additional treatment with long storage sixth day. The highest water levels at treatment with 10% microcapsule addition of storage time first day. Results of analysis of variance showed that additional of layang fish microcapsule did not give significantly different effect of water content on brownies while cold storage at 5 °C give significantly different effect of water content on brownies. The association between duration of storage at 5 °C to a moisture content of brownies shows that the longer shelf, then decreasing water content of brownies on treatment 10%, 20% and 30% of microcapsule. The longer the storage time of the water binding capacity will decrease with increasing amounts of free water. Analysis of Honestly Significant Difference showed that the level of water in storage long first day and third day at a temperature of 5 °C were not significantly different, but were significantly different on sixth day. Lowest brownies water content was found on the sixth day of storage that is 28.12%. Based on the analysis honestly significant difference indicates that the storage of the sixth day is the best treatment because it produces the lowest water content

**Brownies Carbohydrate Concentration**

Carbohydrates play an important role in human life. Carbohydrates (mainly starch) is one of the sources of human food are cheap, and provide around 40-75% of energy intake. Carbohydrates serve as an energy reserve in the human body in the form of glycogen, and as a source of fiber needed by the human body. Carbohydrates provide energy value of 4 kcal/gram (Kusnandar, 2010). The analysis showed that the average value of carbohydrate levels ranged from 33.04% to 43.89%. Highest levels of carbohydrate brownies obtained on providing long microcapsule 10% with 6 days storage at cold temperatures 5 °C. While lowest carbohydrate obtained in treatment of microcapsule 30% at the first day (H1B3) storage. The longer the storage of the declining levels of carbohydrates, this is due to the carbohydrates contained in the brownies will be degraded or hydrolyzed. Results of analysis of variance showed that microcapsule additional give significantly different effect on levels of carbohydrate brownies. Similarly, the longer the treatment of cold storage at a temperature of 5 °C showed a significant effect on levels of

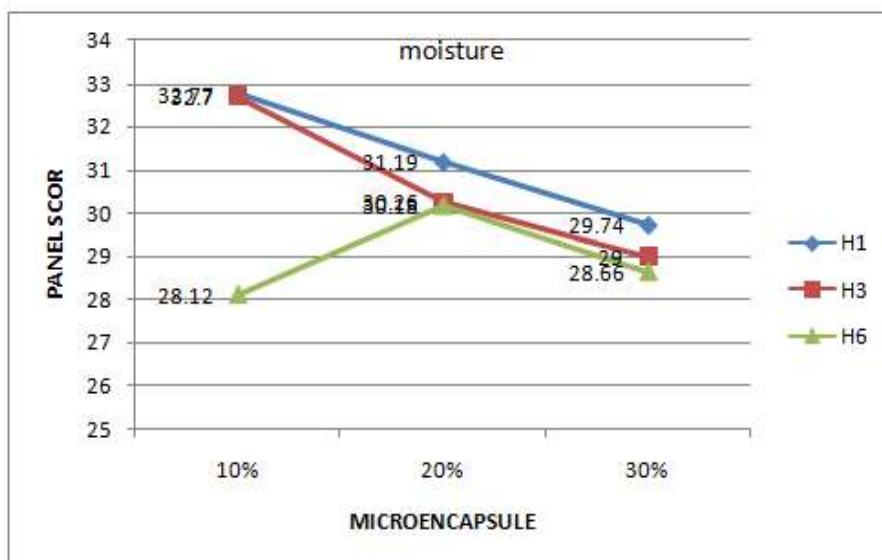


Figure 1. Relationship between Comparative Microcapsule and Long Cold Storage at 5 °C Against Brownies Moisture

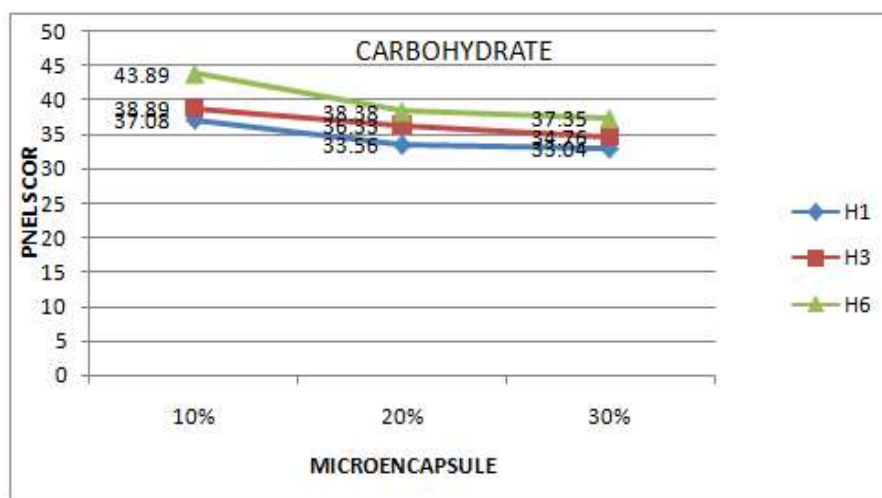


Figure 2. Relationship Between Microcapsule Comparison and long Cold Storage at 5 °C toward for Carbohydrate levels of Brownies

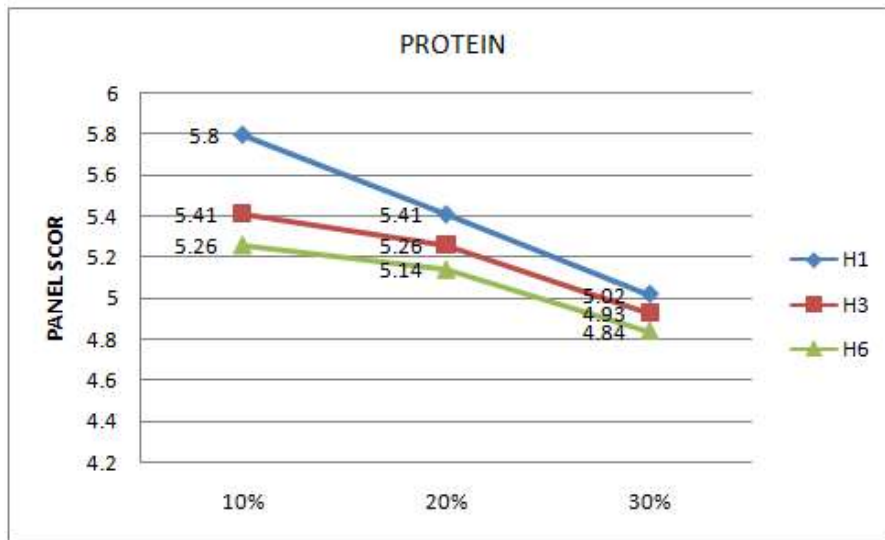


Figure 3. Relationship Between Comparison Microcapsule And long Cold Storage 5 °C toward the levels of Brownies Protein

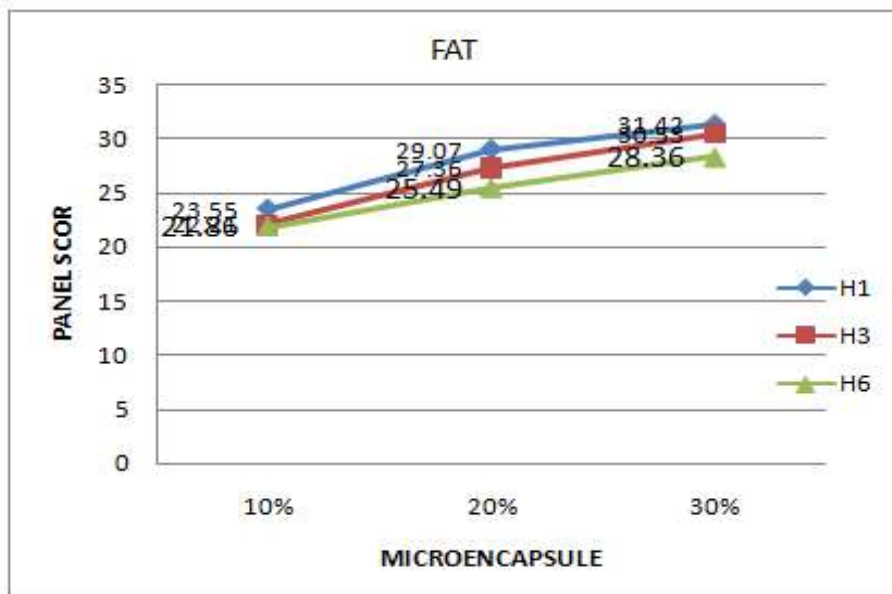


Figure 4. Relationship Between Microcapsule Comparison And long of Cold Storageat 5 °C to levels of Brownies Fat

carbohydrate brownies. In Figure 2, shows that the longer storage at 5 °C then brownies carbohydrate decline.

**Brownies Protein Concentration**

The average value of the protein content ranged from 4.84% to 5.80%. The highest protein content of brownies obtained at addition microcapsule 10% on the first day (H1B1) while the lowest levels of the protein was found in treatment 30% with long storage sixth day at cold temperatures 5 °C. The longer the storage then decreased levels of protein brownies. This is because the protein contained in the brownies will be degraded or hydrolyzed. Results of analysis of variance showed that the treatment mikroenkapsul addition give significantly different effect on the brownies protein content, as well as the treatment of cold storage at 5 °C significantly different effect on protein content brownies. Figure 3 shows that the longer a cold storage at 5 °C, then decreased levels of protein.

This is due to the freezing occurs when protein denaturation and discharge at the time thawing. Results of Honestly Significant Difference analysis showed that the protein content of 10% microcapsule treatment was significantly different from microcapsule treatment 20% and 30%. Based on the HSD analysis it was concluded that microcapsule additional treatment 10% is the best treatment with the highest protein content of 5.49%. Beside that, HSD analysis showed that the protein content significantly difference on the cold storage at 5 °C of the treatment firsth day, third day and sixth day. The best treatment is storage firsth day- because it provides the highest levels of brownies protein 5.41%.

**Levels of Brownies Fat**

The average value of the fat content ranged from 21.86% to 31.42%. The highest levels of brownies fat obtained at microcapsule 30% on the cold storage 5 °C for firsth day. While the lowest fat content was found in treatment of

microcapsule 10 percent with cold storage at 5 °C for sixth day. The higher the concentration of microcapsule then increasing levels of fat, this is due to the fat contained in the brownies will be increased from the oil contained in microcapsule. Results of variance analysis, showed that the addition of microcapsule give the significantly different effect on fat content of brownies. Similarly, treatment of cold storage at 5 °C significantly different effect on fat content of brownies.

### Levels of Brownies Ash

Ash content depicting the many minerals that are not burned into a substance evaporates. The amount of ash content in the product is suspected because of the raw material used from animal material. According to Sudarmadji (1989), that the foods from animal contain high levels of ash because contain some minerals such as calcium, iron and phosphorus. The analysis showed that the average value of brownies ash content ranged from 0.78% to 0.86%. The highest ash content obtained in 10% additional treatment of microcapsule with long storage sixth day at 5 °C. The lowest ash content in the treatment of 20% with the long storage first day at 5 °C. Results of analysis of variance showed that the effect of the microcapsule addition and treatment of duration of storage at 5 °C to the ash content was not significantly different. Figure 5 shows that the longer the storage at 5 °C and the higher concentration of microcapsule, then decreasing ash content of brownies.

### Conclusions

1. The yield of the method (Bligh and Dyer) is 4.05% and the method (Steam and Press) is 3.70%. The quality parameters namely peroxide, avidin and total oxidation included in IFOMA standard. While omega-3 (EPA and DHA) in the method (Bligh and Dyer) respectively of 26.9 mg/g and 16.10 mg/g and methods (steam and press) respectively 4.77 mg/g and 15.29 mg/g
2. Microcapsule yield ranged from 46.86 to 68.90%. The highest yield of 68.90% with the composition of omega 3 EPA in microcapsule is 25.76 mg/g, increase 5.3 times from 4.86 mg/g before crystallization. DHA 17.67 mg/g, an increase of 1.07 times from 16.45 mg/g before crystallization
3. The composition of Omega-3 on the brownies with the microcapsule addition 10%, 20% and 30% produce EPA 12.15 mg/g (10%), 13.86 mg/g (20%) and 18.82 mg/g (30%) mikroenkapsul, whereas DHA 8.39 mg / g (10%), 9.36 mg / g (20%) and 13 mg / g to 30%. Based on the scoring analysis of proximate parameters obtained the microcapsule additional treatment 10% and the cold storage at 5 °C on first day gives the best results compared to the other treatments.

### Suggestions

Based on the findings of this study, it is recommended to do research on the effectiveness of microcapsule on the growth and health of human. Besides, it is necessary to comparing study the effectiveness of functional materials of vegetable oils and laying fish oil.

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