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Research Article

Fatty acid composition of marine microalgae in Indonesia

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ABSTRACT. Marine microalgae are a good source of fatty acids vital for marine organisms. Diversity of marine microalgae in Indonesia could be exploited for marine aquaculture and bio-fuel production. However, basic information pertaining to its fatty acid content is vital prior to any initiative aimed at developing this resource. The fatty acid composition of Indonesian marine microalgae is dominated by saturated fatty acids (SFA) instead of mono unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA). In addition, the fatty acid composition of Class Bacillariophyceae is dominated by C16:0 palmitic acid (38.87-47.68%); C16:1 palmitolic acid (0.5-18.61%); C18:2 linoleic acid (1.34-9.65 %); and C18:3 linolenic acid (0.02-25.31%). The majority fatty acid composition of class Cyanophyceae is C16:0 palmitic acid (47.33-66.05%); C18:0 stearic acid (9.78-14.16 %); C16:1 palmitolic acid (4.14-12.19%) and C18:1 oleic acid (5.31-24.85%). The fatty acid composition of Prasinophyceae is C16:0 palmitic acid (48.42-53.49%) and C18:0 stearic acid (9.16-12.20%). The fatty acid composition of Nannochloropsis (Eustigmatophyceae) is dominated by C16:0 (32.19%); C16:1 (22.4%); C18:0 (14.38%); and C20:0 (Eicosanoic) 13.4%. In addition, *Spirulina platensis* contained the highest amount of palmitic acid C16:0 (66.05 %) belonging to SFA.

Keywords: Microalgae, fatty acid, aquaculture.

INTRODUCTION

Marine microalgae is an important component of the marine flora and it is an essential primary producer that supports carbon footprint in the marine ecosystem. The food pyramid in the marine ecosystem is constructed based on microalgae. Apart from being an important source of food, these tiny plants could be harvested to extract their lipids and used as bio-fuel (Qin, 2005; Schenk *et al.*, 2008).

Indonesia has a vast marine ecosystem supported by hundreds of islands. Hence, it is not surprising that there is a wide diversity of microalgae in these waters. The prokaryotic groups comprise Cyanophyta and Prochlorophyta, and the eukaryophyta groups are made up of Chlorophyta (green algae), Chromatophyta, Chrysophyta and Dinophyta (Tomas, 1997).

Recently, intensification of microalgae based bio-fuel research in the green industry has triggered the search for microalgae strains that could produce high percentage of lipids. An ideal microalgae strain is expected to produce high percentage of lipids in the shortest time. If such a strain is obtained, it will reduce the production cost and make this alternative source of bio-fuel a viable option (Hu *et al.*, 2008; Pratoomyot *et al.*, 2005; Qin, 2005).

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Recently, most research into efficient microalgae oil production is being done by the private sector but they have failed to obtain a good strain of microalgae that need certain specifications such as faster growth-rate compared to terrestrial crops and high lipid content. The difficulties in efficient bio-fuel production from microalgae lie in finding microalgae strain with a high lipid content, and a fast growth rate that is not too difficult to harvest and a cost effective cultivation system (Pratoomyot *et al.*, 2005).

Use of microalgae as bio-fuel is considered a viable option as (a) microalgae can produce more lipid per acre than soybeans and other oil seed crops, (b) microalgae can grow quicker compared to terrestrial plants, (c) mass cultivation of microalgae is possible since it needs less water compared to terrestrial crops, and (d) microalgae cultivation could be potentially more cost effective than conventional farming (Andersen, 2005). However, one major disadvantage of microalgae for bio-fuel production is the low biomass concentration in the microalgae culture due to limited light penetration, which in combination with the small size of microalgae cells makes harvesting of algae biomass relatively costly.

There are some microalgae of which their nucleic acid and lipid content have been used to produce bio-fuel. Strains like *Scenedesmus*, *Chlorella vulgaris*, *Tetraselmis* sp. and *Spirulina* sp. are some common ones (Becker, 1995). Furthermore, many microalgae experts have examined lipid content in some microalgae such as *Botryococcus braunii*, *Isochrysis galbana*, *Tetraselmis chui* and *T. suecica* (Thompson *et al.*, 1992; Qin, 2005). Most importantly, microalgae have the potential to be used as a continuous source of bio-fuel (Qin, 2005; Chisti, 2007; Chisti & Yan, 2011).

Recently, most research on microalgae have focused on industrial aquaculture as rotifer, dapnia, artemia and copepod feed which are fed to late larvae and juvenile crustacean, bivalve, gastropod and fish. A number of microalgae have been studied intensively for their good nutritional quality, high lipid content and fatty acids profile and composition (Brown *et al.*, 1997; Brown, 2004; Reitan *et al.*, 1997; Volkman *et al.*, 1980, 1989, 1991). The purpose of this research is to study the fatty acids profile and composition of selected marine microalgae that are not only used as live feed for aquaculture in Indonesia, but also for selected microalgae that have potential for bio-diesel.

MATERIALS AND METHODS

Microalgae strains

Marine microalgae used in this investigation was obtained from The Research Institute for Marine Fisheries at Lampung in Indonesia. Strains used were *Nitzschia* sp., *Thalassiosira* sp., *Skeletonema costatum*, *Chaetoceros calcitrans* (Bacillariophyceae), *Spirulina platensis*, *Oscillatoria* sp. (Cyanophyceae), *Nannochloropsis oculata* (Eustigmatophyceae), *Porphyridium cruentum* (Rhodophyceae), *Tetraselmis suecica* and *Isochrysis galbana* (Prasinophyceae).

Algal cultivation and harvest

All of the microalgae were cultivated in 100 liter containers using F/2 and Walne as the culture's medium (Andersen, 2005) with initial cell density of 1.0 x 10^6 cell L^{-1} (Table 1). The microalgae culture was illuminated through a 40 watt/m^2 fluorescent light for 24 hours continuously. Microalgae was harvested at a stationary phase for every replicate. The biomass of microalgae was harvested at a centrifugation of 3000 rpm for 30 minutes. After that, the microalgae sample was freezer dried and stored at -50ºC until analysis.

Fatty acid analysis

At the stationary phase, microalgae were harvested by vacuum pump and centrifugation. Fatty acids were analysed by the GC (Gas Chromatography) method at a Research Laboratory (LPPT-UGM) at the University of Gajah Mada, Yogyakarta, Indonesia.

Table 1. The conditions and medium used for culture microalgae.

Microalgae	Media	Temp.(ºC)	Salinity (ppt.)	pH
Class Bacillariophyceae				
Nitzschia sp.	F/2	27-30	35-40	(7-8)
Chaetoceros gracilis	F/2	28-30	30-40	(7-8)
Thalassiosira sp.	F/2	27-31	30-31	(7-8)
Skeletonema costatum	F/2	28-30	20-25	(7-8)
Class Chlorophyceae				
Chlorella sp.	Walne	30-33	30-31	(7-8)
Dunaliella salina	Walne	29-30	30-31	(7-8)
Class Prasinophyceae				
Tetraselmis suecica	Walne	29-30	30-39	(7-8)
Isochrysis galbana	Walne	28-30	30-32	(7-8)
Class Eustigmatophyceae				
Nannochloropsis oculata	Walne	27-30	30-35	(7-8)
Class Cyanophyceae				
Spirulina platensis	F/2	28-31	28-32	(7-8)
Oscillatoria sp.	F/2	28-30	33-37	(7-8)
Class Rhodophyceae				
Porphyridium cruentum	F/2	28-31	25-28	(7-8)

Data Analysis

Data was analysed in detail to describe and explain fatty acids content, profile and composition of microalgae, used as feedstock for aquaculture in Indonesia.

RESULTS

Based on results, the fatty acids compositions of microalgae used in Indonesia as feedstock for aquaculture are shown in Table 2. The major fatty acids of all of microalgae in this research were palmitic acid (C16:0); stearic acid (C18:0) belonging to Saturated Fatty Acids (SFA); and palmitolic acid (C16:1) belonging to Mono Unsaturated Fatty Acids (MUFA) (Table 2).

Cyanophyceae (*Spirulina platensis* and *Oscillatoria* sp.) had high amounts of fatty acids in the form of palmitic acids (C16:0) which had values of 66.05 ± 2.36% and 47.33 ± 7.76%, respectively. *Spirulina platensis* had high amount of saturated fatty acids (81.99 ± 3.91%), low mono unsaturated fatty acid (9.45 ± 1.07%) and poly-unsaturated fatty acids (7.39 ± 0.66%). On the other hand, *Oscillatoria* sp. had high SFA (58.92 ± 9.27%), MUFA (37.04 ± 2.70%) and PUFA only 2.56 ± 0.58%.

The fatty acids profile of *Tetraselmis suecica* and *Isochrysis galbana* were dominated by palmitic acid C16:0 with 53.49 ± 1.55% and 48.42 ± 0.62%, respectively. In contrast, *Isochrysis galbana* had fatty acid percentage profile of linoleic acid C18:2 with 8.67 ± 1.10% and linoleic acid C18:3 with 11.57 ± 0.41% and the sum of PUFA was 22.21 ± 2.41% (Table 2).

The fatty acids profile of *Chlorella* sp. (Chlorophyceae) in this research were palmitic acid C16:0 (30.99 ± 2.55%), Eicosanoic (Arachidic) C20:0 (17.85 ± 1.12%) and Palmitolic acid C16:1 (25.65 ± 2.58%). In this study, the PUFA content of *Chlorella* sp. was very low (Table 2). *Nannochloropsis oculata* (class Eustigmatophyceae) was dominated by palmitic acid C16:0 with 32.19 ± 1.04% and palmitolic C16:1 with 22.4 ± 0.98%.

Table 2. Fatty acids composition of microalgae *Spirulina platensis, Oscillatoria* sp., *Chlorella* sp., *Tetraselmis suecica, Isochrysis galbana* and *Nannochloropsis oculata.*

Fatty Acids		Microalgae					
		Spirulina platensis	*Oscillatoria* sp.	*Chlorella* sp.	*Tetraselmis* sp.	*Isochrysis* sp.	*Nannochloropsis* sp.
Saturated Fatty Acid (SFA)							
C12:0	Dodecanoic (Lauric)	0.39± 0.06	0.31 ± 0.09	0.61 ± 0.13	1.67 ± 0.20	2.84 ± 0.12	0.56 ± 0.10
C14:0	Tetradecanoic (Myristic Acid)	1.01 ±0.02	0	8.62 ± 0.78	8.59 ± 1.51	11.88 ± 0.02	9.33 ± 0.95
C16:0	Palmitic Acid	66.05 ± 2.36	47.33 ± 7.76	30.99 ± 2.55	53.49 ± 1.55	48.42 ± 0.62	32.19 ± 1.04
C18:0	Stearic Acid	14.16 ± 1.32	9.78 ± 1.33	9.00 ± 0.50	12.2 ± 0.57	9.16 ± 1.08	14.58 ± 1.46
C20:0	Eicosanoic (Arachidic)	0.32 ± 0.13	0.42 ± 0.04	17.85 ± 1.12	1.71 ± 0.08	0.36 ± 0.10	13.4 ± 0.99
C22:0	Docosanoic (behenic acid)	0.06 ± 0.01	0.93 ± 0.04	0.10 ± 0.03	0.48 ± 0.06	1.48 ± 0.84	0.08 ± 0.02
C24:0	Tetracosanoic (Lignoceric)	0	0.15 ± 0.02	0.23 ± 0.02	0.14 ± 0.01	0.05 ± 0.03	0.11 ± 0.05
Total SFA		81.99 ± 3.91	58.92 ± 9.27	67.4 ± 0.06	78.28 ± 3.76	74.14 ± 4.70	70.25 ± 0.49
Mono Unsaturated Fatty Acid (MUFA)							
C16:1	Palmitolic Acid	4.14 ± 0.44	12.19 ± 0.70	25.68 ± 2.58	4.85 ± 1.00	2.42 ± 0.73	22.4 ± 0.98
C18:1	Oleic Acid	5.31 ± 0.64	24.85 ± 2.06	0.37 ± 0.02	7.54 ± 0.55	0.18 ± 0.02	1.29 ± 0.42
Total MUFA		9.45 ± 1.07	37.04 ± 2.70	26.05 ± 2.59	12.39 ± 0.50	2.6 ± 0.75	23.69 ± 1.05
Poly Unsaturated Fatty Acid (PUFA)							
C18:2	Linoleic Acid	4.55 ± 0.19	1.82 ± 0.70	0.96 ± 0.01	4.79 ± 0.17	8.67 ± 1.10	0.61 ± 0.13
C18:3	Linolenic Acid	2.62 ± 0.45	0.49 ± 0.03	0.05 ± 0.01	2.22 ± 0.23	11.57 ± 0.41	0.04 ± 0.01
C18:4	Octadecatetraenoic						
C20:5ω3	Eicosapentaenoic Acid (EPA)	0.22 ± 0.04	0.16 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	1.97 ± 0.90	0.32 ± 0.11
C22:6	Docosapentaenoic Acid	0	0.09 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0	0.02 ± 0.01
Total PUFA		7.39 ± 0.66	2.56 ± 0.58	1.19 ± 0.03	7.24 ± 0.41	22.21±2.41	0.99 ± 0.05

All microalgae belonging to class Bacillariophyceae had high amount of fatty acid profile in palmitic acid with a range of 38.87 - 47.68 % (Table 3). Fatty acids profile of *Nitzschia* sp. were palmitic acid C16:0 (38.87 ± 1.04%), palmitolic C16:1 (18.61 ± 1.01%), linoleic acid C18:2 (9.65 ± 1.02%) and linolenic acid C18:3 (2.26 ± 0.93%). Compared with other microalgae, the highest linolenic acid C18:3 was found in *Skeletonema costatum* with 25.31 ± 1.15%. Moreover, the highest value of PUFAs was also found in the *Skeletonema costatum* (27.86 ± 1.61%) and *Isochrysis* sp. (22.21 ± 2.41%). The highest of palmitic acid percentage was found in *Chaetoceros calcitran* (47.68 ± 1.07%). However, this microalgae had a low amount of PUFA (1.87 ± 0.20%) and MUFA (13.61 ± 1.59%).

DISCUSSION

Poly unsaturated fatty acids content in *Isochryis* sp. and *Skeletonema costatum* were higher than in other species of microalgae. This result confers with the findings of Vairappan & Ang (2008) that outdoor cultured *Isochrysis* sp. had 26% more polyunsaturated fatty acids (PUFAs). Enrichment POME (Palm Oil Mill Effluent) to media of *Isochrysis* sp. with photobioreactor and outdoor culture system can not only increase cell density but also produce cell content of PUFA that are important for the development of marine biota (Vairappan & Ang, 2008).

The profile of long-chain omega-3 fatty acids including α-linolenic acid (ALA, 18 carbons and three double bonds), eicosapentaenoic acid (EPA, 20 carbons and 5 double bonds) and docosahexaenoic acid (DHA, 22 carbons and 6 double bonds) were found in *Isochrysis* sp. (Table 2.). In PUFAs profile, the highest procentage of α-linolenic acid (C18:3) was found in *Skeletonema costatum* (25.31 ± 1.15%) and *Isochrysis* sp. (11.57 ± 0.41%). This result is in line with findings of Ryckebosch *et al.* (2012) that some marine microalgae are producers of omega-3 long chain PUFA and are potential alternative live feed for marine organisms culture. The highest procentage of α-linolenic acid (C18:3), one of long-chain omega-3 fatty acids in *Skeletonema costatum* is influenced by environmental factors such as irradiance and carbon (Guiheneuf *et al.*, 2008).

Spirulina platensis and *Oscillatoria* sp. were shown to contain high percentage of C16:0 and were low in poly unsaturated fatty acids (PUFA). This result is in line with findings of Orcutt *et al.* (1986) and Pratoomyot *et al.* (2005) that most Cyanophyceae have very few lipid and their fatty acids composition fall into SFA. A high level of fatty acid C16:0 and low level of PUFA are common in bacteria and blue-green microalgae (Cyanophyceae), especially unicellular blue green microalgae (Orcutt *et al.*, 1986; Piorreck *et al.*, 1984; Pratoomyot *et al.*, 2005).

Based on the result, *Chlorella* sp. contained quite high readings of palmitic acid (30.99 ± 2.5%) and eicosanoic acid (17.85 ± 1.1 %) belonging to SFA and palmitolic acid (25.68 ± 2.58%) belonging to MUFA. The PUFA content in *Chlorella* sp. was very low. This result was similar to the marine *Chlorella* sp. reported by Brown *et al.* (1997) and Pratoomyot *et al.* (2005). In contrast, according to the findings of Otles & Pire (2001), oleic acid (MUFA) in *Chlorella* has a value of 17.62-19.71% and alpha linolenic (ALA) C18:3(w-3) (PUFA) has a value of 13.81-15.87%.

In this study, palmitic acid (C16:0) in *Tetraselmis* sp. was higher than reported by Pratoomyot *et al.* (2005). In contrast, according to Pratoomyot *et al.* (2005), total content of PUFA in *Tetraselmis* sp. is 38.38 % and contains linolenic acid by as much as 16.17%. Furthermore, according to Brown (2004), *Tetraselmis suecica* did not accumulate lipid, but probably accumulated photosynthetic product in the form of carbohydrates.

Nannochloropsis oculata (class Eustigmatophyceae) had Palmitic acid C16:0 content with a value of 32.19 ± 1.04% and dominated by palmitolic C16:1 (22.4 ± 0.98%). This result fits with Hu & Gao (2006) that the predominant fatty acids of *Nannochloropsis* sp. were palmitic acid (16:0), palmitolic acid (16:1)

Table 3. Fatty acids composition of micoalgae *Nitzchia* sp., *Thalassiosira* sp., *Chaetoceros calcitran, Skeletonema costatum* and *Porphyridium cruentum*.

Fatty Acids		Microalgae				
		Nitzschia sp.	*Thalassiosira* sp.	*Chaetoceros calcitran*	*Skeletonema costatum*	*Porphyridium cruentum*
Saturated Fatty Acid (SFA)						
C12:0	Dodecanoic (Lauric)	0.49 ± 0.10	6.83 ± 3.51	2.01 ± 0.97	2.05 ± 0.06	0.28 ± 0.01
C14:0	Tetradecanoic (Myristic Acid)	0.05 ± 0.02	16.56 ± 9.10	3.17 ± 0.67	9.34 ± 1.01	0.46 ± 0.01
C16:0	Palmitic Acid	38.87 ± 1.04	46.01 ± 16.11	47.68 ± 1.07	43.34 ± 1.05	67.99 ± 1.00
C18:0	Stearic Acid	9.14 ± 1.05	0.52 ± 4.64	4.6 ± 0.77	5.11 ± 0.56	3.08 ± 0.77
C20:0	Eicosanoic (Arachidic)	0.1 ± 0.06	2.42 ± 1.46	9.97 ± 1.02	1.23 ± 0.43	1.16 ± 0.13
C22:0	Docosanoic (behenic acid)	4.1 ± 1.11	1.73 ± 1.62	1.83 ± 0.92	3.67 ± 0.50	0
C24:0	Tetracosanoic (Lignoceric)	13.6 ± 1.05	2.99 ± 6.09	4.1 ± 0.20	4.69 ± 0.98	0.07 ± 0.01
Total SFA		66.35 ± 4.43	77.06 ± 26.36	73.36 ± 1.74	69.43 ± 2.42	73.04 ± 1.60
Mono Unsaturated Fatty Acid (MUFA)						
C16:1	Palmitolic Acid	18.61 ± 1.01	0.5 ± 9.6	6.94 ± 1.02	1.17 ± 0.82	0.88 ± 0.01
C18:1	Oleic Acid	2.14 ± 0.36	5.4 ± 2.32	6.67 ± 0.58	0.62 ± 0.61	6.8 ± 0.1
Total MUFA		20.75 ± 1.38	5.9 ± 8.56	13.61 ± 1.59	1.79 ± 1.43	7.68 ± 0.11
Poly Unsaturated Fatty Acid (PUFA)						
C18:2	Linoleic Acid	9.65 ± 1.02	1.34 ± 4.45	1.6 ± 0.10	1.41 ± 0.59	1.81 ± 0.02
C18:3	Linolenic Acid	2.26 ± 0.93	7.93 ± 3.30	0.02 ± 0.01	25.31 ± 1.15	0.29 ± 0.02
C18:4	Octadecatetraenoic					
C20:5n-3	Eicosapentaenoic Acid (EPA)	0	1.24 ± 1.15	0.1 ± 0.08	1.14 ± 0.12	0.07 ± 0.01
C22:6	Docosapentaenoic Acid	0	0	0.15 ± 0.01	0	0
Total PUFA		11.91 ± 1.94	10.51 ± 4.18	1.87 ± 0.20	27.86 ± 1.61	2.17 ± 0.05

and EPA (20:5n-3). In contrast, in this study, *Nannochloropsis oculata* had low EPA (0.19 ± 0.01 %). In this research, the culture medium of *N. oculata* had a temperature range of 27-30°C. The higher palmitic acid was probably due to the high temperature in the culture medium. A research gave evidence that increased temperature enhanced the percentage of palmitic acid, but the EPA decreased (Hu & Gao, 2006). Apart from high temperature in the media used for culture of *Chlorella* sp, nutrient limitation is a precursor for Acetil-CoA carboxylase enzyme to produce biosynthesis lipid (Schenk *et al.*, 2008).

Fatty acids profile of *Nitzschia* sp. and *Chaetoceros calcitran* were dominated by palmitic acid C16:0 and palmitolic (C16:1). *Skeletonema costatum* was dominated by linolenic acid C18:3 (25.31 ± 1.15%). In contrast to the other microalgae belonging to class Bacillariophyceae, *Skleletonema costatum* had high value of total PUFA (27.86%). This result is higher compared to those reported for diatom *Nitzschia* spp., *Thalassiosira* spp., *Chaetoceros* spp. (Thompson *et al.*, 1996; Brown *et al.*, 1997; Pratoomyot *et al.*, 2005). In this study, diatoms contained higher fatty acid than the others species, because the structure of diatoms cells accumulate lipids while environmental factors can affect growth and fatty acid profiles of diatom (Guiheneuf *et al.*, 2008).

The highest palmitic acid percentage was found in *Porphyridium cruentum* (class Rhodophyceae) which had a value of 67.99 ± 1.00 %. The second highest was found in *Spirulina platensis* (66.05 ± 2.36 %). In this research, all microalgae investigated were similar in fatty acids profile, but differed in amount of fatty acids level. The high percentage of fatty acids content of microalgae is due to the harvest of microalgae at stationary phase. Based on a study by Pratoomyot *et al.* (2005, the percentage of fatty acid content at the stationary phase was higher than at the exponential phase. Due to limited nutrition at the stationary phase, cell division gradually decreased and the cells began to store products (Hoek & Mann, 1995). Additionally, limitation of nutrition such as nitrate, phosphate and silicate of the culture medium can increase Acetil CoA carboxylase enzyme, which is a precursor for making lipid in microalgae (Schenk *et al.*, 2008).

In addition, the fatty acids profile of microalgae was dominated by palmitic acids C16 :0; stearic acids C18 :0; palmitolic acids C16 :1; linoleic acids C18 :2 and linolenic acids C18 :3 (Table 2). This fits with research by Harrington (1986) that all plants oil used for biodiesel production have to contain fatty acids C16 and C18.

In this study, most of the microalgae investigated have similar fatty acid profile, but the percentage content of fatty acid for each microalgae is very different. This mainly depends on the strain used and culture conditions (Volkman *et al.*, 1989; Renaud *et al.*, 2002; Tzovenis *et al.*, 2003). All of the microalgae have the same fatty acid profile in chain C16 and C18. Palmitat fatty acid (C16:0) is a predominant fatty acid in most microalgae culture in this research. In conclusion, *Spirulina platensis, Porphyridium cruentum* and *Tetraselmis* sp. were identified as having high palmitat fatty acid content. Thus, these microalgae could be potential candidates for production of bio-diesel. On the other hand, *Spirulina platensis*, *Tetraselmis* sp., *Nitzschia* sp., *Thalassiosira* sp., *Isochrysis galbana* and *Skeletonema costatum* have higher PUFA content compared to other microalgae. It could be concluded that *Isochrysis galbana* and *Skeletonema costatum* are suitable as a quality live feed for aquaculture. These species of microalgae could serve as good nutritional sources of PUFA for aquaculture.

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