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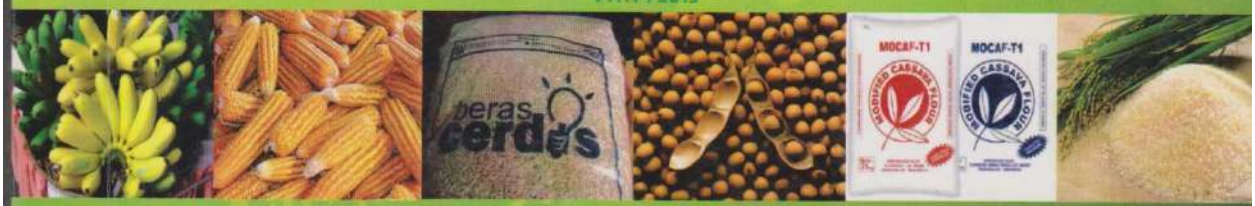

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DAFTAR ISI

EFFECT OF SODIUM HEXAMETHAPOSPHATE ON DIETARY FIBER-ANTIOXIDANT PROERTIES EXTRACTED FROM GREEN CINCAU (*Premnaoblongifolia*Merr) LEAFS

[Pengaruh Sodium HexamethapospatterhadapkarakteristikSeratPangan-Antioksidan yang DiekstraksidariDaunCincauHijau (*Premnaoblongifolia*Merr)]

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Abstract

Dietary fiber and antioxidants have received a lot of attention due to their ability to modulate health status. The aim of this research was to investigate physico-chemical and some functional properties and antioxidant capacity of dietary fiber-antioxidant extracted from Green cincauleafs (*Premnaoblongifolia* Merr) using sodium hexamethaphosphate (SHMP) sequestering agent solution in different concentration.The results showed that increasing of SHMP concentrations increased the yield of dietary fiber-antioxidantand their pectin content. Degree of esterification (DE) of the pectin was in the range of 40 to 50 percent whereas increasing of SHMP increase the pectin DE. Water solubility and oil holding capacity were not affected by SHMP concentration but their viscosity reached maximum value when the SHMP concentration was 0.125%. All of the extract had free radicals scavenging activity but it was not affected by SHMP concentration. As the extract ofgreen cincu leafs contains both high pectin concentration and antioxidant activity, it is worthwhile to develop it as a dietary fiber-antioxidant ingredient.

Key words: Antioxidant, dietary fiber, Green cincau, *Premnaoblongifolia* Merr.

Abstrak

Serat pangan dan antioksidan mendapat banyak perhatian karena potensinya dalam memelihara kesehatan. Tujuan penelitian ini adalah untuk mengkaji berbagai sifat fisikokimia, sifat fungsional dan aktivitas antioksidan serat pangan antioksidan yang diekstraksi dari daun cincau hijau (Premnaoblongifolia Merr) menggunakan larutan sodium heksametapospat (SHMP) dalam berbagai konsentrasi. Hasil penelitian menunjukkan bahwa peningkatan konsentrasi SHMP meningkatkan randemen ekstrak dan kadar pektinnya. Derajat esterifikasi (DE) pectin berkisar antara 40 hingga 50 persen di mana peningkatan kadar SHMP menyebabkan peningkatan DE. Kelarutan dalam air dan kapasitas penyerapan minyak tidak dipengaruhi penambahan SHMP, tetapi nilai viskositas mencapai maksimal pada penambahan SHMP 0,125%. Semua ekstrak memiliki kemampuan menetralkan radikal bebas tetapi tidak dipengaruhi oleh penambahan SHMP. Karena estrak dari daun cincau hijau mengandung pektin dan antioksidan, maka akan sangat bermanfaat untuk mengembangkan ekstrak tersebut menjadi ingredient serat pangan antioksidan.

***Kata kunci:** Antioksidan, serat pangan, cincau hijau, Premnaoblongifolia* Merr

INTRODUCTION

Dietary fiber and antioxidants have received a lot of attention due to their ability to modulate health status. Dietary fiber and antioxidants that are not digested in the small intestine will reach the colon and be metabolized by colon microbiota(Manach et al., 2005, Saura-Calixto et al., 2010). At the same time, antioxidants will modulate the colon microbial population via their action as antibacterials or substrates, and metabolism of antioxidants will produce further simple metabolites with varied activity(Saura-Calixto et al., 2010, Bellion et

al., 2008). Previous research indicates dietary fiber induces colonic metabolism of the phenolic compounds linked/trapped by dietary fiber (Saura-Calixto et al., 2010). The microbial metabolites of dietary fiber or antioxidant have beneficial health effect through their action in the colon or when absorbed, they are able to modulate health status.

Green cincau (*Premnaoblongifolia*Merr) is a tropical plant belonging to the Verbenaceae family. Publications on this plant as dietary fiber source are limited but preliminary research on the leaf extract, such as their ability to induce cell-mediated immune responses *in vitro* (Nurdin et al., 2003) or their laxative effect (Nurdin, 2007) indicates promising results. The extract also effectively induced the growth of lactic acid bacteria in the colon compared to cellulose (Nurdin, 2007) and have antioxidant activity as well as interesting characteristics (high water holding capacity and viscosity) (Nurdin et al., 2005). As the extract from *Premnaoblongifolia*Merr.leaves contain both high pectin levels in an unpurified form and phenolic compounds, it is worthwhile to develop it asa dietary fiber-antioxidant ingredient.

Green cincau leaves arecommonlyextractedwithwatertopreparea traditional drink containingpolysaccharideforminggel. The polysaccharides are mainly pectin (19.71%) especially low methoxyl pectin physically bound by divalent cationin the middle lamella(Artha, 2001;Nurdin et al., 2005). Applying sequestering agent in the extraction process of this stuff should increase their extractability. Due to functional and technological properties should be considered as criteria for choosing desirable dietary fiber sources (Lopez et al., 1996), therefore, our research was aimed to characterize green cincauextracts extracted using SHMP sequestering agent solution in different concentration.

MATERIALS AND METHODS

Green Cincau Leave materials and the Extraction Process

Green cincau leafs (PremnaoblongifoliaMerr.) were collected from traditional farmers in Lampung-Indonesia. The fresh leafs were dried in oven at 50°C to get dry leaves (water content around 12%), and ground to obtain dry cincau powder(Nurdin et al., 2005).. Extracts were obtained from extraction of 5% dry cincau leaf powder using hot water containing 0.000, 0.125, 0.250, 0.375, 0.500, 0.625 % (w/v) of SHMP followed by oven drying at 50°C, grinding and filtering to get 80 mesh powder.

Characterization of the Dietary Fiber-Antioxidant extract

Dietary fibre-antioxidant extract (dried extracts powder) were analyzed to determine their total pectin content (Apriantonoet al., 1989)and their radical scavenging activity (Przybiylski, 1998). Functional properties of the fibre concentrate that were measured including water holding capacity, water solubility, oil holding capacity, and viscosity (Widayatet.al, 2003).

Determinatio of Degree of Esterification of the Pectin

Pectin was isolated from dried extract powder through ethanol precipitation. Degree of esterification of the pectin was measured following procedure reported by Soebrata (1999).

Statistical analysis. The data was subjected to one-way ANOVA. The treatment means were compared using Least Significant Different (LSD) test and the differences were considered to be significant if the associated *P* value was 0.05.

RESULTS AND DISCUSION

Effect of SHMP concentration on yield and fungsional characteristic of Dietary Fiber-Antioxidant extracted from green cincau leaves

SHMP increased extractable stuffs from green cincau leafs (Fig. 1). The yield of dried extract was almost doubled when the concentration of SHMP increase from 0.00% to 0.625%. The fresh extract is forming gel with pectin as the main gel forming component (Artha, 2001). It was suggested SHMP chelated metal ion bound with pectin in middle lamella of green cincau leafs (Thakur et al., 1997). Due to the metals strengthening the cell wall structure integrity were lost, polysaccharides including pectin composing cell wall of green cincau leafs are easier to extract. Therefore, more metals ion were chelated, more cell wall components were extracted.

Figure 1. Effect of SHMP concentration on yield of Dietary Fiber-Antioxidant extracted from green cincau leaves. The bars represent the mean, and the lines are SEM of three replicates. Data points denoted by different superscripts (letters on the bar) differ significantly with $p < 0.05$.

Pectin content of the extract was increased by increasing of SHMP concentration, but the SHMP effect was not dose dependent (Fig 2.). SHMP effect was not observed when its level was 0,250% and 0,375 % or 0,500 and 0,675. Pectin is bound withmetal ion in the middle lamella of plant cell walls (Thakuret al., 1997). As the metal was chelated, the cells wall of green cincrau leafs will be softened, and their pectin content is more extractable. Eventhough the effect of SHMP on pectin content was not dose dependence, the positive correlation trend is observed (Fig 2), whereas addition of 0,625 % SHMP resulted in about 100% increment of pectin content.

Figure 2. Effect of SHMP concentration on pectin content of Dietary Fiber-Antioxidant extracted from green cincau leaves. The bars represent the mean, and the lines are SEM of three replicates. Data points denoted by different superscripts (letters on the bar) differ significantly with $p < 0.05$

Pectin is a polysaccharide composed of D-galacturonic acid units linked by a (1-4) glycosodic bond. The gelling characteristics of pectin are determined by the molecular size and degree of esterification (DE) (Thakur et al., 1997). Commonly, pectin from different sources has different properties due to variations in this parameter. Increasing level of SHMP increased the DE of pectin extracted from green cincau leafs (Fig 3).Commercial pectin has DE values in range of 60 – 75% for high methoxyl pectin and 20 – 40% for low methoxyl pectin (Sriamornsak, 2003), therefore, all the pectin extracted using SHMP can not be categorized as commercial pectin. A recent study showed the structures of oligosaccharides fractionated from pectin had a significant impact on fermentation by human fecal bacteria, with greatest bifidogenic activity seen with the low-molecular-weight oligoarabinosidesand oligogalactosides(Onumpai et al., 2011). Previously, Dongowski et al. (2000) found that low-methoxyl pectin was fermented faster than high-methoxylpectins in vivo and in vitro.

Figure 3. Effect of SHMP concentration on degree of esterification of pectin extracted from green cincau leaves. The bars represent the mean, and the lines are SEM of three replicates. Data points denoted by different superscripts (letters on the bar) differ significantly with $p < 0.05$

Oil holding capacity refers to the capacity of a fiber to bind oil or fat. The OHC of the extracts were not affected by SHMP concentration (Tabel 1) and ranged between 0.88 g oil/g extract for product extracted using 0,250% SHMP and 1,190 g oil/g extract for product extracted using 0,000% SHMP solution. Similar results were found by Figuerola et al. (2005) for dietary fiber from grape, apple and lemon (1,41, 1,00, 1,39 g oil/g dry matter, respectively). Grigelmo-Miguel and Martin-Belloso (1999) reported similar OHC value (0,90 – 1,30 g oil/g fibers) for dietary fiber rich pectin from orange and suggested the application of

the product for clouding agent in beverages, thickener and gelling agent as well as binder, texturizer and low calorie bulk ingredient.

Table 1. Effect of SHMP concentration on functional characteristic of dietary fiber-antioxidant extracted from green cincau leaves.

Viscosity is a physicochemical property of dietary fibers associated with their polysaccharides. Viscous dietary fibers had several metabolic health benefits through their effect on reducing of absorption rate of nutrition in small intestine (Jenkins et al., 2004). Our research indicated that SHMP concentration affected the viscosity of dietary antioxidant from green cincau leafs (Table 1). The highest viscosity was achieved when the extract was produced using 0,125 % SHMP (616,67cPs), then it dropped significantly when the SHMP concentration increased. Similar pattern on viscosity was seen when the solution for extraction containing citric acid as a sequestering agent (Nurdin et al., 2005). These authors suggested that metal ions and pH of solution were involved in the viscosity changes of green cincau leafs extracts.

Solubility of the dietary fiber-antioxidant extracts were about 5,00 % (w/v), not depend on how high the SHMP was applied (Table 1). This solubility is much higher than those reported by Nurdin et al. (2005) when the extraction process using citric acid solution (ranged between 1,78 and 2,00 % w/v). Therefore, applying of SHMP as a chelating agent in dietary fiber-antioxidant extraction process from green cincau leafs is more beneficence than applying citric acid in term of their potentiality for beverage dietary fiber fortification.

Effect of SHMP concentration on radical scavenging activity of Dietary Fiber-Antioxidant extracted from green cincau leafs

The antioxidant activity was estimated by scavenging activity of the extracts against 2, 2-Diphenyl-1-Picryhydrazyl (DPPH) radical. Five percent of all extracts show antioxidant activity (Fig 4) in the range from 42,70 to 52,08%, but the activity is was not affected by SHMP concentration. Previously, Nurdin et al. (2005) showed that green cincau extract produced through extraction using citric acid solution had radical scavenging activity in the range from 42,08 to 47,29%. These values are lower than those reported by Daou and Zhang (2011) for radical scavenging activity of dietary fiber derived from defatted rice brand whereas at 5,00% concentration, their scavenging activity were more than 60,00 and 80,00% for soluble and insoluble dietary fiber, respectively. Green cincau extracts contain alkaloids, saponins, phenol hydroquinones, molisch, benedict and tannins (Aryudhani, 2011). Therefore, it suggested that the phytochemical compounds in the cincau exhibited antioxidant activity against the radicals.

Figure 4. Effect of SHMP concentration onRadical scavenging activity of Dietary Fiber-Antioxidant extracted from green cincau leafs. The bars represent the mean, and the lines are SEM of three replicates.

The role of dietary fiber on the accessibility and bioavailability of antioxidants in fruit and vegetables has been proposed (Sauro-Calixto, 2010;Palafox-Carlos et al., 2011). Dietary fiber can inhibit antioxidant absorption in the intestine through their action as antioxidant entrapper during digestion in the intestine, or when the antioxidants are bound to other macromolecules and need enzyme hydrolysis to be absorbed, dietary fiber that forms matrices in the chime can restrict the enzymes action. Furthermore, when antioxidants are not absorbed in the intestine, they reach the colon with dietary fiber and will be metabolized by colon microbial where their metabolites may modulate human health (Palafox-Carlos et al., 2011).

Our results indicate that green cincau extract contains both dietary fiber and antioxidant compounds, therefore, they may exhibits combination of physiological effects of dietary fiber and antioxidant. Several researches have been reported regarding the efficacy of dietary fiber-antioxidant on disease prevention. Sanches-Tena et al. (2013) found that red grape pomace rich in dietary fiber and proanthocyanidin greatly reduce

interstinaltumorigenesis in the $Apc^{Min/+}$ mouse model indicated by decreasing of total number of polyps by 76%. Mechanisms by which the red grape pomace reduced the incidence of intestinal tumorigenesis are mainly through induction of a G cycle arrest and downregulation of genes related to the immune respone and inflammation (Sanchez-Tena et al., 2013). Previously, Jimenez et al. (2008) showed that grape product rich in dietary fiber and antioxidant (5,25 g of dietary fiber and 1400 mg of polyphenols) significantly reduced lipid profile and blood pressure of adult nonsmoker after ingested the product for 16 wks. These authors suggested that the grape product was more effective than oat fiber or psyllium dietary fibers, probably due to the combined effect of dietary fiber and antioxidants (Jimenez et al., 2008). Further researches are deeded to elucidate whether the dietary fiber-antioxidants from green cincau leafs has beneficial health effect or not.

CONCLUSION

SHMP has significant effect on the dietary fiber-antioxidant production, especially on the yield, pectin concentration, pectin DE and viscosity of the extract. Increasing of SHMP concentrations increased the yield of dietary fiber-antioxidant and their pectin content. Degree of esterification (DE) of the pectin was in the range of 40 to 50 percent whereas increasing of SHMP increase the pectin DE. Water solubility and oil holding capacity were not affected by SHMP concentration but their viscosity reached maximum value when the SHMP concentration was 0.125%. All of the extract had free radicals scavenging activity. As the extract from *Premnaoblongifolia*Merr. leafs contain both high pectin concentration and antioxidant activity, it is worthwhile to develop it as a dietary fiber-antioxidant ingredient.

REFERENCES

Artha, N. 2001.IsolasidanKarakterisasiSifatFungsionalKomponenPembentuk Gel CincauHijau (*Cycleabarbata*L. Miers).Disertasi.IPB. Bogor.

Aryudhani N. 2011. Mechanism of antitumor activity of powdered leaf green grass jelly (Premna blongifolia Merr.) In mice transplanted c3h breast tumor cells *http://repository.ipb.ac.id/handle/123456789/51752. Downloaded 24 August 2012.*

Bellion P, Hofmann T, Pool-Zobel B L, Will F, Dietrich H, Knaup B, Richling E, Baum M, Eisenbrand G, Janzowski C. 2008. Antioxidant effectiveness of phenolic apple juice extracts and their gut fermentation products in the human colon carcinoma cell line caco-2. *Journal of Agricultural and Food Chemistry.* 56: 6310-6317.

Daou C, Zhang H. 2011. Physico-chemical Properties and Antioxidant Activities of Dietary Fiber Derived from Defatted Rice Bran.Advance Journal of Food Science and Technology 3(5): 339-347.

Dongowski G, Lorenz A, Anger H. 2000. Degradation of Pectins with Different Degrees of Esterification by *Bacteroidesthetaiotaomicron*Isolated from Human Gut Flora. Appl. Environ. Microbiol. 66(4): 1321-1327.

Figuerola F, Hurtado ML, Estevez AM, Chiffelle I, Asenjo F. 2005. Fibre Concentrates from Apple Pomace and Citrus Peel as Potential Fibre Sources for Food Enrichment. Food Chem. **91,** 395-401.

Grigelmo-Miguel N, Martin-Belloso O. 1998. Characterization of dietary fiber from orange juice extraction. Food Research International. 31(5):355-361.

Hidayat, B., Surfiana, Widodo, Sarono, Kalsum, Y.R., danMuslihudin, N. 2003. Optimasi Proses ProduksidanKarakteristikSeratmakananLarut Air Sempurna (*invisible dietary fiber*) dari Bran Gandum (*wheat bran*) sertaaplikasinyapadaprodukbiskuitdanminumanringan (soft drink)

diperkayaseratmakananalami. LaporanBogasariNugraha. PoliteknikPertanian. Bandar Lampung.

Jenkins DJA, Marchie A, Augustin LSA, Ros E, Kendall CWC.Viscous dietary Fibre and Metabolic Effects.Clinical Nutrition Supplement. 1:39-49.

Jiménez JP, Serrano J, Tabernero M, Arranz S, Díaz-Rubio ME, García-Diz L, Goñi I, Saura-Calixto F. 2008. Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. Nutrition. 7-8: 646-653.

Lopez G, Ros G, Rincon F, Periago J, Martinez MC, Ortuno J. 1996. Relationship between physical and hydration properties of soluble and insoluble fiber of artichoke.J. of Agricultural and Food Chemistry.**44:** 2773-2778

Manach C, Mazur A, Scalbert, A. 2005. Polyphenols and prevention of cardiovascular diseases. *Current Opinion in Lipidology*. 16: 77-84.

Nurdin SU, Zuidar SA, Suharyono. 2005. Dried extract from green cincau leaves as potential fibre sources for food enrichment. *African Crop Science Conference Proceedings*. 7: 655-658.

Nurdin S U, Hwang J K, Hung P. 2003. Effect of Green Cincau Leaf (*Premna oblongifolia* Merr.) Water Extracts on Cytokines Production in Whole Cell Culture of Mouse Splenocytes. *Indonesian Food and Nutrition Progress*. 10: 70-74.

Nurdin SU. 2007. Evaluasi Efek Laksatif dan Fermentabilitas Komponen Pembentuk Gel Cincau Hijau (*Premna oblongifolia* Merr.). *Teknologi dan Industri Pangan*. 18: 10-16.

Onumpai C, Kolida S, Bonnine E, Rastall RA. 2011. Microbial utilization and selectivity of pectin fractions with various structures. *Applied and Environmental Microbiology*. 77: 5747-5754.

Palafox-Carlos h, Ayala-Zavala JF, Gonzales-Aguilar GA. 2011. The Role of Dietary Fiber in the Bioaccessibility and Bioavailability of Fruit and Vegetable Antioxidants. J. Food Sci. 76(1): 1-10.

Przybiylski R, Lee YC, Eskin,Nia M. 1998. Antioxidantand Radical Scavenging Activities of Buckwheat Seed Component.JAOCS.**75:**1595-1601

Sánchez-Tena S, Lizárraga D, Miranda A, Vinardell MP, García-García F, Dopazo J, Torres JL, Saura-Calixto F, Capellà G, Cascante M. 2013. Grape antioxidant dietary fiber inhibits intestinal polyposis in ApcMin/+ mice: relation to cell cycle and immune response. Carcinogenesis.DOI:10.1093/carcin/bgt140

Saura-Calixto F, Perez-Jimenez J, TourinoS, Serrano J, Fuguet E, Torres JL, Goni I. 2010. Proanthocyanidin metabolites associated with dietary fibre from in vitro colonic fermentation and proanthocyanidin metabolites in human plasma. *Molecular Nutrition and Food Researc*. 54: 939-946.

Soebrata BM. 1999. IsolasidanPenentuanSifatPektinLabu Siam (*Sechiumedule*).Buletin Kimia. 4:1-9.

Thakur BR, Singh R K, Handa A K. 1997. Chemistry and uses of pectin--a review. *Critical reviews in food science and nutrition*. 37: 47-73.

FIGURES AND TABLE

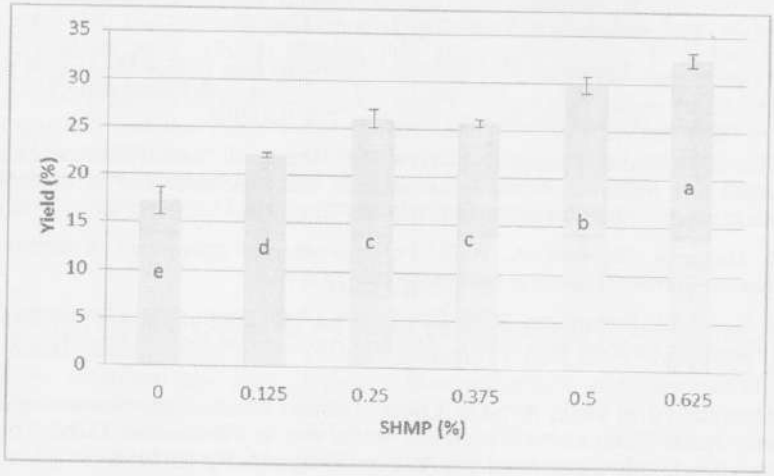
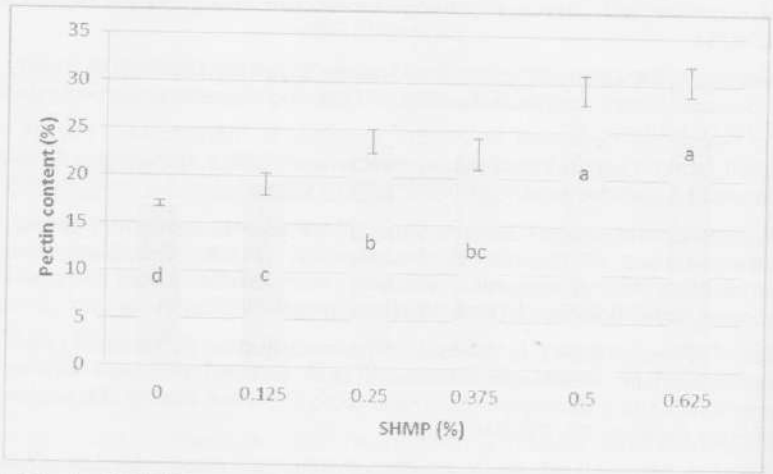


Figure 1. Effect of SHMP concentration on yield of Dietary Fiber-Antioxidant extracted from green cincau leaves. The bars represent the mean, and the lines are SEM of three replicates. Data points denoted by different superscripts (letters on the bar) differ significantly with $p < 0.05$.



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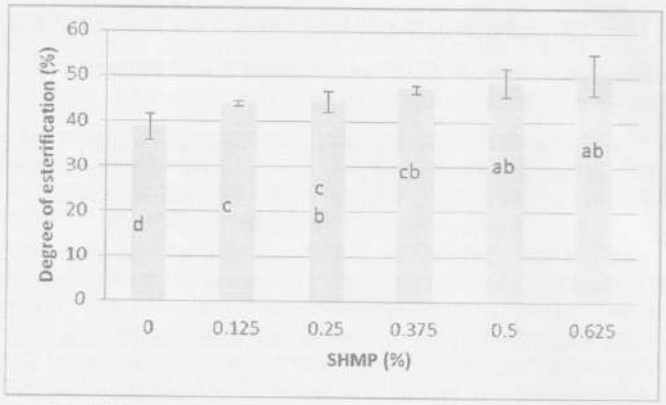

Figure 3. Effect of SHMP concentration on degree of esterification of pectin extracted from green cincau leaves. The bars represent the mean, and the lines are SEM of three replicates. Data points denoted by different superscripts (letters on the bar) differ significantly with $p < 0.05$

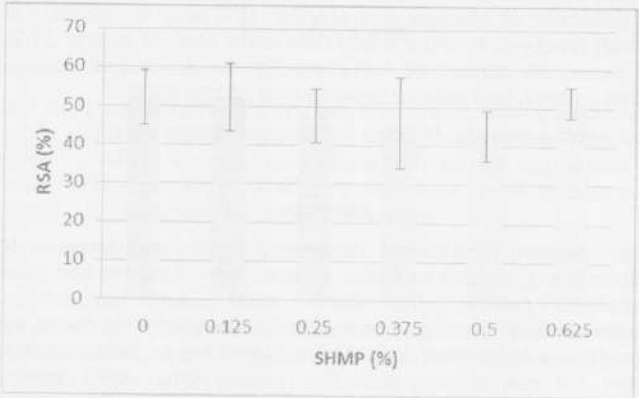

Figure 4. Effect of SHMP concentration onRadical scavenging activity of Dietary Fiber-Antioxidant extracted from green cincau leafs. The bars represent the mean, and the lines are SEM of three replicates.

Table 1. Effect of SHMP concentration on functional characteristic of dietary fiber-antioxidant extracted from green cincau leaves.

Functional Characteristic	SHMP concentration (%)[a]					
	0.000	0.125	0.250	0.375	0.500	0.675
OHC ($g_{oil}/g_{extract}$)	1.19	0.95	0.88	0.97	0.94	0.92
Viscosity (cPs)	208.33a	616.67b	266.67a	290.00 a	191.67a	148.33a
WS (%w/v)	5.50	5.33	5.17	5.50	5.50	5.67

[a] Values are mean of tri replicates. Means across rows with a different superscript are statistically significantly different at $p \leq 0.05$.