

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

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[Pengaruh Sodium Hexamethapospat terhadap karakteristik Serat Pangan-Antioksidan yang
Diekstraksi dari Daun Cincau Hijau (*Premna oblongifolia* 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 (*Premna oblongifolia* 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-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 but it was not affected by SHMP concentration. As the extract of green cinca leaves 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, *Premna oblongifolia* Merr.

ABSTRAK

Serat pangan dan antioksidan mendapat banyak perhatian karena potensinya dalam memelihara kesehatan. Tujuan penelitian ini adalah untuk mengkaji berbagai sifat fisiko kimia, sifat fungsional dan aktivitas antioksidan serat pangan antioksidan yang diekstraksi dari daun cincau hijau (*Premna oblongifolia* 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 dimana peningkatan kadar SHMP menyebabkan peningkatan DE. Kelarutan dalam air dan kapasitas penyerapan minyak tidak dipengaruhi penambahan SHMP, tetapi nilai viskositas menyampai maksimal pada penambahan SHMP 0,125%. Semua ekstrak memiliki kemampuan menetralkan radikal bebas tetapi tidak dipengaruhi oleh penambahan SHMP. Karena ekstrak dari daun cincau hijau mengandung pectin dan antioksidan, maka akan sangat bermanfaat untuk mengembangkan ekstrak tersebut menjadi ingredient serat pangan antioksidan.

Kata kunci: Antioksidan, serat pangan, cincau hijau, *Premna oblongifolia* Merr

INTRODUCTION

33 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.

2 Green cincau (*Premna oblongifolia* 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 *Premna oblongifolia* Merr. leaves contain both high pectin levels in an unpurified form and phenolic compounds, it is worthwhile to develop it as a dietary fiber-antioxidant ingredient.

Green cincau leaves are commonly extracted with water to prepare a traditional drink containing polysaccharide forming gel. The polysaccharides are mainly pectin (19.71%) especially low methoxyl pectin physically bound by divalent cation in 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 cincau extracts extracted using SHMP sequestering agent solution in different concentration.

MATERIALS AND METHODS

Green Cincau Leave materials and the Extraction Process

2 Green cincau leaves (*Premna oblongifolia* Merr.) were collected from traditional farmers in Lampung-6 Indonesia. The fresh leaves 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 (Apriantono et al., 1989) and their radical scavenging activity (Przybylski, 1998). Functional properties of the fibre concentrate that were measured including water holding capacity, water solubility, oil holding capacity, and viscosity (Hidayat et.al, 2003). 18

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).

26 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. 10

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 leaves (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 leaves (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 leaves are easier to extract. Therefore, more metals ion were chelated, more cell wall components were extracted.

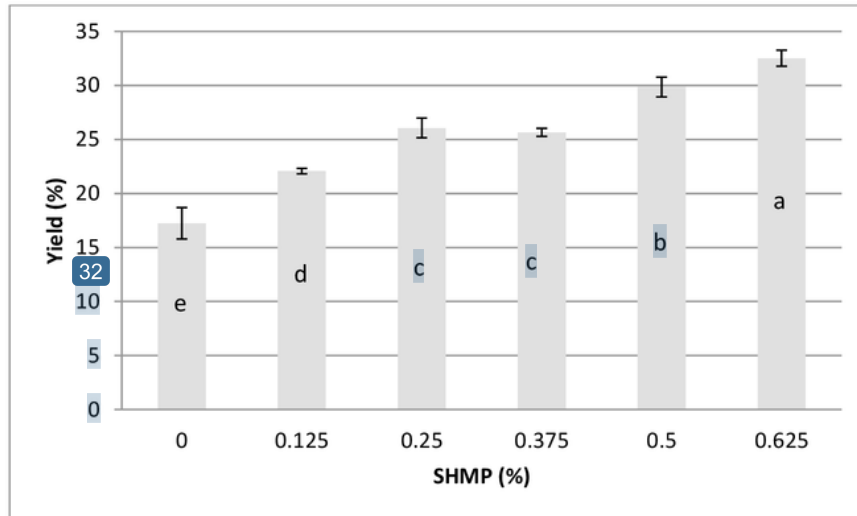


Figure 1 Effect of SHMP concentration on yield of Dietary Fiber-Antioxidant extracted from green cincau leaves. The bars represent the mean, and the lines are SEM of three replicates. Data points denoted by different superscripts (letters on the bar) differ significantly with $p < 0.05$.

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 with metal ion in the middle lamella of plant cell walls (Thakur et al., 1997). As the metal was chelated, the cells wall of green cincau leaves 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.

Pectin is a polysaccharide composed of D-galacturonic acid units linked by a (1-4) glycosidic 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 leaves (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 oligoarabinosides and oligogalactosides (Onumpai et al., 2011). Previously, Dongowski et al. (2000) found that low-methoxyl pectin was fermented faster than high-methoxyl pectins in vivo and in vitro.

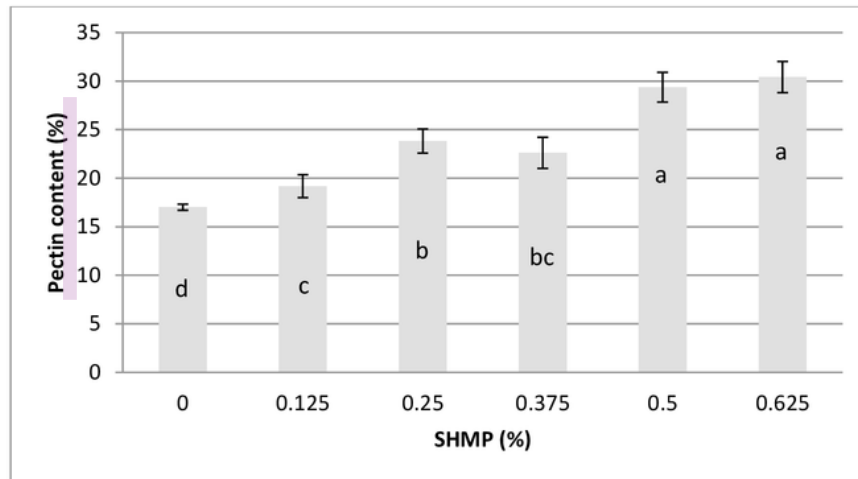


Figure 2. Effect of SHMP concentration on pectin content of Dietary Fiber-Antioxidant extracted from green cincau leaves. The bars represent the mean, and the lines are SEM of three replicates. Data points denoted by different superscripts (letters on the bar) differ significantly with $p < 0.05$

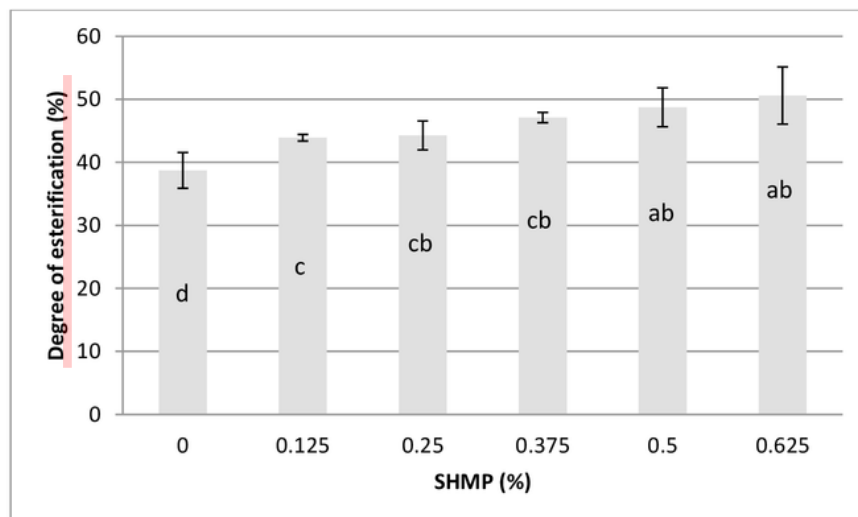


Figure 1 Effect of SHMP concentration on degree of esterification of pectin extracted from green cincau leaves. The bars represent the mean, and the lines are SEM of three replicates. Data points denoted by different superscripts (letters on the bar) differ significantly with $p < 0.05$

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.

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	290.00a	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 $p \leq 0.05$.

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 (616,67 cPs), 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 leaves

The antioxidant activity was estimated by scavenging activity of the extracts against 2, 2-Diphenyl-1-Picrylhydrazyl (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.

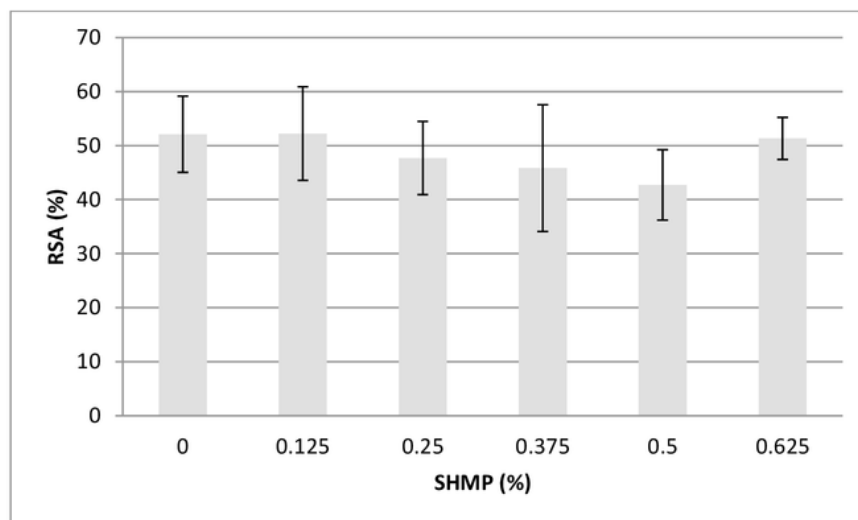


Figure 4. Effect of SHMP concentration on Radical scavenging activity of Dietary Fiber-Antioxidant extracted from green cincau leaves. The bars represent the mean, and the lines are SEM of three replicates.

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 exhibit 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 intestinal tumorigenesis 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 response 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 needed to elucidate whether the dietary fiber-antioxidants from green cincau leaves 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 *Premna oblongifolia* Merr. leaves contain both high pectin concentration and antioxidant activity, it is worthwhile to develop it as a dietary fiber-antioxidant ingredient.

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