

# **Production of New Generation of Protein Antioxidant from *Gnetumgnemon*Seed (Gg-AOP) as Nutraceutical Resource**

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## **EXECUTIVE SUMMARY**

Reactive oxygen species (ROS) have effects on many substances in the human body, such as fatty acids, proteins and DNA. Generation of reactive oxygen species or free radicals such as superoxide, hydroxyl radical and hydrogen peroxide during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress. Oxidative stress plays a role in heart diseases, malaria, neurodegenerative diseases, AIDS, cancer and in the aging process. All organisms have antioxidant systems that are able to control and counter the onslaught of free radical mediated oxidative damage. Therefore, dietary sources have been recognized as safer and effective antioxidants in the context of their efficiency and non-toxicity. The intake of fruits and vegetables containing high amounts of antioxidant nutraceuticals has been associated with the balance of the free radicals or antioxidants status, which helps to minimize the oxidative stress in the body and to reduce the risks of diseases. Recently, the use of natural product has attracted particular interest.

As an archaic living fossil of the *Gnetumgnemon*, L (Gnataceae) is one of the oldest species of tree, with great anti-adversity ability, which has existed on the earth for two hundred million years. *Gnetumgnemon* (melinjo), L (Gnataceae) is cultivated in Indonesia, Malaysia and other south-east Asian islands for its seeds and is used as food in Indonesia. This tree may be considered as a suitable source of functional food, nutraceutical food supplement and their high bioavailability. The characters potency of *Gnetumgnemon* (melinjo) seed component such as starch, lipid, protein and phenolics/flavonoids as a nutraceutical sources for food supplement were very

limited. In line with this, the main our research is to studies of potency and development of functional components from melinjo seed (*Gnetumgnemon*) as nutraceutical food supplement.

Extraction and Isolation, Twenty five grams of sample seed were ground in a food processor and the resulting meal was extracted with 50 mL of an ice-cold extraction distilled water for 2-4 hours at 4°C. Protein isolated was hydrolysed with trypsin, pepsin and alcalase on their optimal hydrolysis condition. The degree of hydrolysis was determined using trinitrobenzenesulphonic acid (TNBS) reaction (Alder-Nissen, 1979). ABTS radical scavenging activities of Gg-AOP fractions were determined as described by siswoyoet *al.* with slight modification.

Scavenging radical activities of melinjo (*Gnetumgnemon*) protein isolate (Gg-PI) hydrolysates prepared by three enzymes (alcalase, trypsin and pepsin). The antioxidant activities and protective effect against oxidative DNA damage of Gg-PI hydrolysates were investigated. Alcalasehydrolysates exhibited the highest hydroxyl radical-scavenging activity (IC<sub>50</sub> 1.74 mg mL<sup>-1</sup>) (*P* < 0.05). Compared with other two hydrolysates, the hydrolysates obtained by alcalase had the most abundant <3-kDa fractions. In addition, below 3-kDa fractions of alcalasehydrolysates showed the highest antioxidant activities and protective effects against DNA damage through both scavenging hydroxyl radicals, which was probably because of the increase in several antioxidant amino acids, such as His, Met, Cys, Tyr and Phe, as well as the hydrophobic amino acids. The results suggested that enzymatic hydrolysis could be used as an effective technique to produce high value-added peptides products from *Gnetumgnemon* seed.

*Keywords: Antioxidant, Protein, Gnetumgnemon, Nutraceutical, Production*

References ;

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## RINGKASAN

Kebutuhan akan bahan makanan tambahan (supplement) untuk mempertahankan atau meningkatkan sistem fisiologis pada tubuh sangat diperlukan terutama ditujukan untuk pencegahan atau pengobatan terhadap suatu penyakit tertentu. Penggunaan senyawa alami biofungsional protein sebagai nutraceutical merupakan suatu pilihan dikarenakan kespesifikanya dalam fungsi fisiologis. Pencarian sumber genetik alami potensial terutama dari tanaman asli Indonesia mulai dikembangkan. *Gnetum Gnemon* (melinjo) adalah tanaman asli Indonesia yang dalam penelitian sebelumnya menunjukkan bahwa protein biji melinjo mempunyai kemampuan sebagai polipeptid antioksidan (free radical scavenging /Gg-AOP) (Siswoyo et al., 2007; Siswoyo et al., 2011) dan potensi aktif menghambat beberapa jenis bakteri dan jamur (Siswoyo et al., 2006) dengan karakter protein yang lebih stabil pada suhu tinggi (Siswoyo et al., 2007). Sumber protein antioksidan baru ini (Gg-AOP) diharapkan pada usulan penelitian ini dapat dimodifikasi secara kimia, fisik dan biologi (enzimatik) sehingga upaya untuk memperoleh dan memproduksi protein generasi baru dari *Gnetum gnemon* dengan aktivitas lebih tinggi optimalisasi produksi secara lebih besar protein aktif yang terkandung dalam biji melinjo, 2) Pengujian *in vitro/vivo* dan sebagian karakterisasi protein aktif hasil produksi, Model produksi protein antihipertensi dengan kondisi suhu dapat dilakukan. Kedepannya kebutuhan akan bahan komersial Nutraceutical Food Supplement berupa protein fungsional generasi baru dapat terpenuhi secara cepat dan tepat. Pada tahun II telah dilakukan pekerjaan kurang lebih sebesar 100% dari seluruh rencana pekerjaan/target, dengan rincian sebagai berikut : 1) re-evaluasi inkubasi 50o C, lama waktu 2-3 jam dengan rasio 0.2% E/S, 4) telah didaftarkan patent dengan judul “Ekstraksi Protein Antioksidan dari Biji Melinjo (*Gnetum gnemon*), Produk dan Kegunaan sebagai Bahan Nutraceutical Food supplement”, dan 6) sebagian hasil penelitian ini telah dipresentasikan diseminar Japanese Society of Cereal Scientists and the 4th Asia Pasific Protein Association (2014) dan telah dipublikasikan dalam jurnal internasional (*Journal of Medical Plant Research*). Hasil penelitian ini diharapkan dapat menghasilkan penemuan baru berupa

metode produksi protein generasi baru dari PI-Gg-AOP dan informasi secara rinci memperoleh protein generasi baru dari Gg-AOP. Inovasi teknologi produksi ini telah didaftarkan untuk dipatenkan dan dipublikasikan secara nasional/international serta produknya dapat digunakan sebagai bahan komersial nutraceutical food Supplement yang dapat diaplikasikan pada bahan pangan atau olahan.

*Keywords: antioksidan, Protein, Gnetum gnemon, melinjo, Nutraceutical, Production*

