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LC-MS/MS profiles and interrelationships between the anti-inflammatory activity, total phenolic content and antioxidant potential of Kalasin 2 cultivar peanut sprout crude extract

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ABSTRACT

Peanut is a potent natural source of phytochemical compounds and is associated with human health. In the present study, we determined the biological activity and chemical constituents of peanuts germinated for 0–9 days. The ungerminated seed coat exhibited the highest antioxidant potential, phenolic compound content and anti-inflammatory activity. During the germination process, on the first germination day, root extract showed the highest antioxidant potential, phenolic compound content and anti-inflammatory activity. The kernel exhibited a low phenolic compound content and antioxidant activity at the early stage of germination and significantly increased after 9 days of germination. Resveratrol increased to 7.19 ± 0.07 μg/g dry weight on the second day of germination. LC-MS/MS showed a variety of phenolic compounds and stilbene derivatives in different parts of germinated peanut. These results suggest that the peanut sprout exerts high anti-inflammatory effects that may be related to the polyphenolic content and antioxidant properties.

1. Introduction

Peanut (*Arachis hypogaea L.*) is considered to be a highly nutritious food due to its high protein content, unsaturated fatty acid content, polyphenolic content, fiber content and bioactive components. Peanuts are rich in polyphenol substances, such as resveratrol. Resveratrol (3,4′,5-trihydroxystilbene) belongs to the stilbene group and displays anti-inflammatory, antioxidative and cytoprotective effects (Malhotra, Bath, & Elbarbry, 2015). Our previous study (article in press) demonstrated that the resveratrol content varies between different peanut cultivars and that the peanut sprout has a higher resveratrol content than the ungerminated peanut kernel, depending on the peanut cultivar. Choi et al., 2015 evaluated the anti-inflammatory activity of the ethanolic extract of peanut sprout, which contains a high level of trans-resveratrol, by assessing its antioxidant activity in both mouse skin and compound 48/80-treated HaCaT cells *in vitro*. Several studies have reported that a wide variety of phenolic substances, such as resveratrol, piceatannol and arachidin-1, can be found in peanut and peanut sprout (Xiong et al., 2014; Hasan, Cha, Bajpai, & Baek, 2013).

The inflammatory process is a defense mechanism to protect cells from injury and infection. The purpose of inflammation is to localize and remove injurious components so that the cell can begin to repair itself. A malfunction of the inflammatory process can result in chronic inflammation, which is involved in many unrelated disorders, such as asthma (Murdoch & Lloyd, 2010), allergies (Francisco et al., 2014) and oxidative stress-related diseases (Reuter, Gupta, Chaturvedi, & Aggarwal, 2010). During inflammation, pro-inflammatory enzymes, such as lipoxygenase (LOX) and cyclooxygenase (COX), are activated (Haeggström & Funk, 2011). These enzymes catalyze the addition of an oxygen molecule to linoleic acid and arachidonic acid to form hydroperoxide products, such as hydroperoxyeicosatetraenoic acids (HPETEs), hydroxyeicosatetraenoic acids (HETEs), leukotrienes and lipoxins. These products act as mediators of the inflammatory process and are involved in the development of numerous inflammatory disorders. There is increased consumer interest in natural products that contain a variety of bioactive nutrients. As nutrients rich in phytochemicals, phenolic compounds and flavonoids are essential for human health, there is increasing interest in health-promoting foods that exhibit pharmacological activities, such as anti-inflammatory, antimicrobial, anticancer and neuroprotection. Several reports suggest that fruits, grains and vegetables exhibit

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anti-inflammatory activities due to their antioxidative potential (Araruna & Carlos, 2010; Alhakmani, Kumar, & Khan, 2013). Tabart et al. (2012) reported that blackcurrant leaf extract exhibited the highest antioxidant and anti-inflammatory capacities, which correlated to the highest total phenolic content. The phenolic compound content and antioxidant capacity of six legumes during germination and found that the phenolic content significantly increased during germination in all legumes. The previous study also revealed that the germinated peanuts and soybeans achieved maximum phenolic compounds and antioxidant capacity (Khang, Dung, Elzaawely, & Xuan, 2016). The phenolic compounds are generally secondary metabolites produced in plants and may thus correspond to antioxidant activity, which confers various biological benefits.

No report thus far has investigated the anti-inflammatory and antioxidant enzymes produced in Kalasin 2 peanut cultivar during the germination period. In this study, we investigated the antioxidant properties, total phenolic compound content, resveratrol content and antioxidant enzyme activities during a 9-day germination period. The relationship between natural antioxidant status and anti-inflammatory activity on the different growth stages was also investigated. The information obtained may promote the economic value of peanut as a health-promoting functional food in the future.

2. Materials and methods

2.1. Chemicals and reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Fluka (Buchs, Germany). Standard compounds of resveratrol, lipoxygenase type I-B from soybean, linoleic acid and Folin-Ciocalteu phenol reagent were purchased from Sigma-Aldrich (St. Louis, USA). HPLC-grade solvents, including water, methanol, acetonitrile, ethyl acetate and formic acid, were purchased from RCL Labscan Limited (Bangkok, Thailand). Potassium persulfate was purchased from Merck (Darmstadt, Germany).

2.2. Peanut sprout extraction

Seeds of Kalasin 2 peanut cultivars were soaked in 0.5% NaCl solution for 30 min and subsequently submerged in tap water for 3 h to rehydrate. Seeds were then germinated in the dark for 15 min in spray tap water with pauses of 15 min for 0–9 days. Ungerminated peanut kernels were discarded. The peanut kernel (0–9 days), root (1–9 days) and seed coat (0–5 days) of the germinated peanut sprouts were collected. Only 0–5 day seed coat samples were collected due to the rapid detachment of the seed coat from peanut kernels during seed imbibition upon the extended germination time. All samples were subsequently dried in an oven at 60 °C. The dried peanut kernel, root and seed coat were pulverized, followed by 80% ethanol extraction for 24 h. Solvent evaporation was carried out on a rotary evaporator at 40 °C and crude extract was collected.

2.3. Total phenolic content assay

Total phenolic content was determined using the Folin-Ciocalteau method. The reaction was initiated with 2 μL crude extract (10 mg/mL) mixed with 50 μL Folin's reagent followed by the addition of 50 μL sodium carbonate (20% w/v) solution. The reaction was incubated for 30 min in the dark at room temperature and absorbance was measured at 765 nm. Gallic acid was used as the standard and the results are expressed as GAE (gallic acid equivalent, μg gallic acid/g dry weight). Each sample was run in triplicate.

2.4. ABTS radical scavenging assay

The ABTS assay is based on the inhibition of the $ABTS^{\cdot+}$ radical cation (2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid)) by the presence of an antioxidant in the crude extract. The assay procedure was modified slightly from that described by Re et al. (1999). Briefly, 7 mM ABTS solution was oxidized with 2.45 mM potassium persulfphate and incubated in the dark at room temperature for 12–16 h to generate the ABTS radical cation ($ABTS^{\cdot+}$) before use. The reaction was initiated by the addition of 2 μL crude extract (10 mg/mL) to 198 μL of the $ABTS^{\cdot+}$ radical. The reaction was mixed immediately and incubated in the dark for 6 min and the OD was measured at 734 nm at room temperature. Measurements were recorded in triplicate and Trolox was used as the standard. The results of the antioxidant assay are expressed as TEAC (Trolox equivalent antioxidant capacity, mM Trolox/g dry weight).

2.5. Antioxidant enzyme activity assay

2.5.1. Superoxide dismutase activity (EC 1.15.1.1) assay

Superoxide dismutase (SOD) activity was measured following the method proposed by Beyer & Fridovich, 1987. The assay is based on the ability of an enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). Peanut sprouts (1.0 g) were homogenized in 6 ml extraction buffer (50 mM sodium phosphate buffer, pH 7.8, 1 mM EDTA and 2% polyvinylpolypyrrolidone). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 0.06 mM EDTA, 5 mM l-methionine, 1 mM NBT, 0.2 mM riboflavin and crude extract. The reaction was initiated by exposure to a 13-W LED lamp for 5 min. The photoreduction of NBT was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% in 1 min under standard conditions.

2.5.2. Catalase activity (EC 1.11.1.6) assay

Catalase (CAT) activity was determined according to the method proposed by Aebi, 1984. Peanut sprouts (1.0 g) were grinded in 6 ml homogenization buffer (50 mM sodium phosphate buffer, pH 7.8, 1 mM EDTA and 2% polyvinylpolypyrrolidone). CAT activity was measured by monitoring the disappearance of H_2O_2 at 240 nm. The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 1 mM EDTA, and 4% H_2O_2. The reaction was initiated by the addition of crude enzyme and the continuous reduction of absorbance at 240 nm was monitored in 10-s intervals over a total of 3 min. One unit of CAT corresponded to the amount of enzyme needed to decompose one micromole of H_2O_2 per minute under standard conditions.

2.5.3. Ascorbate peroxidase activity (EC 1.11.1.11) assay

Ascorbate peroxidase (APX) activity was measured based on the reduction of ascorbate concentration (modified from Nakano & Asada, 1981). The protein crude extract was obtained by homogenizing 1.0 g of peanut sprout tissue in 6 ml extraction buffer (50 mM sodium phosphate buffer, pH 7.8, 1 mM EDTA, 2% polyvinylpolypyrrolidone and 2 mM sodium ascorbate). The oxidation rate of ascorbate was determined in a reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0), 1 mM EDTA, 5 mM sodium ascorbate and 3% H_2O_2. The decrease in absorbance at 290 nm was continuously monitored for 3 minutes. One unit of ascorbate peroxidase activity was defined as the amount of enzyme that oxidized one micromole of ascorbate per minute under standard conditions.