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Effect of Germination on Antioxidant Activity of Peanut Sprouts *(Arachis Hypogaea)*

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Abstract

Peanuts (Arachis hypogaea L.) are plant-based foods that are abundantly available in Indonesia which also have high economic value and are an essential source of protein for many people. In Aceh, peanuts are one of the leading commodities, especially in the districts of Pidie, West Aceh, Aceh Jaya, Nagan Raya, Southwest Aceh and South Aceh. Apart from being rich in protein, it also contains fibre, antioxidants, and other good health components. During germination, proteins, starches, and lipids are metabolised by enzymes. These components are used as building blocks for growth and as a source of energy for respiration. Germination has the advantage of increasing several levels of nutrients, eliminating oligosaccharides that cause flatulence, and increasing several types of vitamins. Previous research reported that soybean germination significantly increased isoflavone levels and antioxidant activity of fresh tempeh flour produced. In this study, the antioxidant activity of peanut sprouts was identified using the DPPH (2,2-diphenyl-1-picryl-hydrazine-hydrate) radical scavenging method in three different germination times. The result shows that the most optimal percentage of antioxidant activity is the 48-hour treatment (88.24%). It also shows that the germination treatment can increase the per cent of antioxidant activity compared to peanuts without germination.

Keywords: Antioxidant activity; Germination; Peanut sprout.

Introduction

Antioxidants are molecules that can prevent damage to cells caused by the oxidation of other molecules in the form of oxidation reactions that will form free radicals and cause chain reactions. This is where the role of antioxidants is to eliminate free radical intermediaries and inhibit oxidation reactions by oxidising themselves. Plants have complex systems of various antioxidants (Mamta Pal et al. l, 2014). Therefore, it is necessary to supplement natural antioxidants derived from food to enhance the body's endogenous antioxidant defence system to reduce the adverse effects of the reaction of oxygen and nitrogen species (Barreiro et al., 2022). For this reason, it is necessary to find other alternatives to overcome these problems. One way is to replace synthetic antioxidants with natural antioxidants, one of which is sourced from nuts.

Nuts are plant foods that are abundantly available in Indonesia. Peanut *(Arachis hypogaea L.)* is Indonesia's second most crucial type of legume after soybean. Unfortunately, few studies have investigated and utilised peanuts in the health sector. Whereas peanuts have great potential to be explored with a protein content of around 25-30%, carbohydrates of 12%, and oil of 40-50%, as well as vitamins (A, B, C, D, E and K), also contain minerals such as calcium, chloride, iron, magnesium, phosphorus, potassium and sulfur (Kusumaputri, 2010). Much evidence supports the health benefits of consuming legumes. Legumes have been reported can significantly lower risks of heart disease, high blood pressure, stroke, and type 2 diabetes (Polak *et al.*, 2015).

Every year the need for peanuts continues to increase in line with the increase in population, the community's nutritional needs, food diversification, and the increasing capacity of Indonesia's feed and food industry. In Aceh, peanuts are one of the leading commodities, especially in the districts of West Aceh, Pidie, Aceh Jaya, Nagan Raya, Southwest Aceh, and South Aceh. The land commonly used by farmers for peanut cultivation is paddy fields and dry land. The available paddy fields and dry lands in Aceh Province are pretty extensive, with 350,515 Ha and 1,223,983 Ha, respectively. The average yield of peanuts in Aceh Province continued to increase from 2013 to 2015, reaching 955 tons, 1,200 tons, and 1,500 tons per year, respectively (BPS, 2014).

The germination process is a complex series of changes in plants' morphology, physiology and biochemistry. Proteins, starches and lipids will be overhauled by enzymes used as building blocks for growth in areas of growing points and as fuel for respiration (Sutopo, 2002). During germination, the reserve material is degraded and used for respiration and the synthesis of new cells before developing the embryo (Vidal-Valverde, 2002). The germination process increases the lysine content of the grain and the amylase activity. Lipase during germination increases the grain's sugar and essential fatty acid content (Chavan & Kadam, 1989). The nutritional value contained in the seeds will change during the germination process. This change in nutritional value can be used to improve the nutritional value of foodstuffs or processed products. Research by Astawan (2020) reported that the soybean germination process had a significant effect ($p<0.05$) on isoflavone levels and the antioxidant activity of fresh tempeh flour produced. This study aimed to investigate germination's effect on peanut sprouts' antioxidant activity.

Methods

This research was conducted at the Laboratory of Nutrition, Department of Nutrition, Teuku Umar University, and the Laboratory of Food Analysis, Faculty of Agriculture, Syiah Kuala University.

Germination process

The germination process was conducted by washing the peanuts and then soaking them for 8 hours, and then the peanuts were rewashed and drained. The clean beans were placed on trays and covered with cloth for germination according to treatment (0, 24, 48, and 72 hours) and every 12 hours. The beans were watered by spraying water.

Extraction by Maceration Method (Istiani, 2010)

A total of 100 g of sprouts were blended to form a paste. The sprout paste was then macerated in 250 ml of 70% ethanol. After 24 hours, the filtrate was collected. The residue was added with 100 ml of 70% ethanol, then macerated for 24 hours, filtered, and the filtrate was collected. The second residue was added with 100 ml of 70% ethanol. The filtrate from the maceration was then concentrated with a rotary evaporator to obtain a thick extract. Then it dried the extract in the oven for 30 minutes at $50^{o}C$ to obtain isolates.

Antioxidant Activity Test with DPPH Method (Brand-Williams et al. (1995) with modification)

DPPH Solution Preparation

The DPPH solution was prepared by weighing 2.5 mg of DPPH crystals and dissolved in 100 mL of methanol. Then the absorbance was measured at a wavelength (λ) of 517 nm as the control absorbance.

Sample Solution Preparation

The test solution was prepared by weighing the extract as much as 1 mg and dissolving it into 2 mL of ethanol to make a test solution with a concentration of 100 ppm. Then 0.1 ml of the sample solution was taken, and 5 ml of DPPH solution was added. The solution was incubated for 30 minutes in a room without light. Furthermore, the visible absorbance was measured at a wavelength of 517 nm as sample absorbance.

Antioxidant activity is expressed in terms of per cent DPPH radical capture and calculated by equation (Yen and Chen, 1995 in Ariani and Hastuti, 2009).
% Antioxidant activity = (1-sample absorbance/ control absorbance x 100%)

Results

Morphology of Peanut Sprout

Germination is one of the stages in the growth of plants that have been widely studied to increase the nutritional value of materials. The morphology of the peanut sprout after germination is shown in Figure 1, which shows that there was radicle growth in peanut sprouts after germination for 24, 48, and 72 hours.

Figure 1. Morphological appearance of peanut sprouts

(a) Germination 24 hours (Length: ± 0.5 cm), (b) Germination 48 hours (Length: ± 1 cm), (c) Germination 72 hours (Length: ± 2 cm)

Table 1. Antioxidant Activity

Treatments	Germination Time (hours)	Antioxidant Activity (%)
Peanuts	0	82.16_a
	24	87.24_c
Peanut Sprouts	48	88.24_d
	72	86.54_b

Table 1 shows that based on germination time, peanut sprouts germinated within 48 hours and had the most optimal percentage of antioxidant activity (88.24%). The results also showed that the antioxidant activity in all peanuts with germination treatment was significantly higher than the per cent antioxidant activity in peanuts without germination treatment.

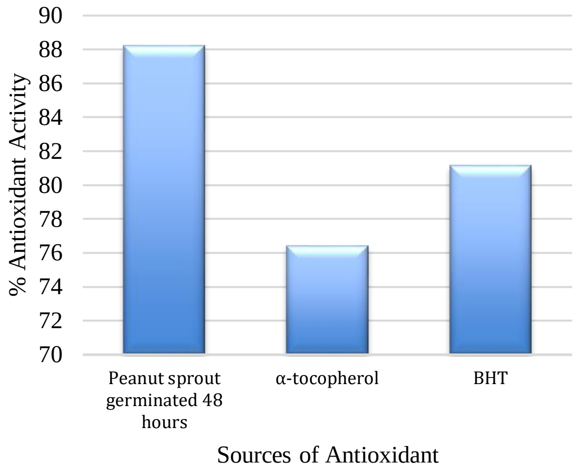

Figure 2. Comparison of Antioxidant Activity of Peanut Sprout with Other Sources of Antioxidant

Figure 2. shows that the antioxidant activity of peanut sprouts germinating in 48 hours was higher than that of alpha-tocopherol as a natural antioxidant and butylated hydroxytoluene (BHT) as an artificial antioxidant.

Discussion

Morphology of Sprout after Germination

An effort to improve the quality of peanuts is through the germination process. Germination has been recognised as an inexpensive process and an effective technology for improving the quality of nuts and seeds. Each type of legume has different characteristics; this will affect the germination process, including the germination time.

The morphology of peanuts after germination (Figure 1.) shows radicle growth in peanut sprouts after germination for 24, 48, and 72 hours. This illustrates that after soaking treatment, there is a process of nutrient breakdown that can activate enzymes. According to Mooy et al. (2021), the soaking process before germination results in the formation or activation of enzymes that increase metabolic activity. The presence of water in the cell activates several enzymes at the beginning of germination. Therefore, radicle formation can occur because enzymes have been produced. Ferdiawan et al. (2019) reported that the germination process is where the radicle extends outward through the seed coat. The stages of germination begin with hydration or imbibition, followed by enzyme activation, embryo growth, and subsequent sprout growth (Shofi, 2017). According to Walter et al. (2000) in Ferdiawan et al. (2019), the pattern of water absorption in the germination process occurs in 3 phases. Phase I: begins with rapid water absorption due to the potential difference between water and seeds. Phase II is characterised by slow water absorption because the seed's water potential and environment are balanced (active seed metabolism takes place). Phase III: is characterised by swelling until the sprout emerges.

Sprouts are young seeds obtained from grain germination with a short growth period. Germination is used to overcome antinutrients or indigestibility that limit the use of legumes as food (Bau, 1997; Mubarak, 2005). During the process, these decrease while nutritional components (e.g., glucose, amino acids, and fatty acids) and polyphenols increase (Ganesan, 2018; Bau, 1997). Germination under abiotic stress conditions, including salinity, drought, low temperature, and illumination, can increase the content of phytochemicals, including phenolics, flavonoids, and melatonin (Dueñas, 2015; Saleh, 2019; Aguilera, 2015; Arnao, 2015). The light exposure provided will also affect the production of bioactive compounds. Phytochemical compounds will increase when germinating seeds in the dark (Guo, 2012; Hung, 2020; Aguilera, 2014).

Antioxidant Activity

The nutritional value and content of legumes become better after going through the germination process. During the germination process in legumes, some of the enzyme systems become active, and changes occur in several nutritional components, including an increase in vitamin C content and protein levels (Shah, 2011). During germination, antioxidant activity can increase along with phenolic compounds content. Due to their chemical structure, phenolic compounds have hydroxyl groups which can act as free radical scavengers (Uchegbu and Ishiwu, 2016). Antioxidants are compounds that can protect foodstuffs by slowing damage, rancidity or discolouration caused by oxidation (Dungir et al., 2012).

Antioxidant activity testing was conducted using the DPPH method. This method was chosen because it is simple, easy, fast, and sensitive and requires only a few samples. DPPH antiradical activity method is a method to screen the antioxidant activity of natural materials (Molyneux, 2004 in Amrun and Umayah, 2007). Antioxidant compounds will react with DPPH radicals through the hydrogen atom donation mechanism and cause the decay of DPPH colour from purple to yellow.

The results of the antioxidant activity of peanut sprout samples are summarised in Table 1. Based on the antioxidant activity test results, the average antioxidant activity from the highest to the lowest is significantly obtained, including peanut sprouts with 48 hours germination, peanut sprouts with 24 hours germination, peanut sprouts with 72 hours germination, and ungerminated peanuts. This explains that the germination time affects the antioxidant activity. There was an increase in antioxidant activity as germination time increased until it reached a maximum of 48 hours and decreased at 72 hours. According to Istiani (2010), this may occur due to the hydrolysis of glycoside compounds into free isoflavone compounds called aglycons by the enzyme β-glucosidase. The decrease in antioxidant activity is also possible due to the further reaction of isoflavone compounds into other derivative compounds whose unknown activities need to be studied more deeply.

The position of peanut sprouts as an antioxidant compared to several existing antioxidants, such as α-tocopherol as a natural antioxidant and BHT as an artificial antioxidant, is shown in Figure 2. Based on Figure 2, it can be seen that the antioxidant activity of peanut sprouts within 48 hours of germination treatment has better antioxidant activity than α-tocopherol and BHT. This proves the potential of peanut sprouts as a natural antioxidant and even higher activity than synthetic antioxidants such as BHT. According to Chang et al. (1977), using certain synthetic antioxidants, such as BHT, can cause adverse effects on consumer health, such as liver, lung, intestinal mucosa, and poisoning. Therefore, the potential of peanut sprouts as a natural antioxidant can be further studied so that it can be applied to functional food products that are beneficial to support public health.

Conclusion

The germination of peanuts with time intervals of 24, 48, and 72 hours produced peanut sprouts with an average growth of 0.5 cm, 1 cm, and 2 cm, respectively. Based on the germination time, the peanut sprouts with the most optimal percentage of antioxidant activity in the 48-hour treatment (88.24%). It also shows that the per cent of antioxidant activity in all peanuts with germination treatment can increase the percentage of antioxidant activity compared to those without germination treatment. It is necessary to pay attention to selecting raw peanut materials with good quality to succeed in the germination process. In addition, environmental humidity, light, and oxygen levels also need to be considered in the germination process. We recommend further study on other bioactive components in peanut sprouts and their application associated with health.

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Author Contribution and Competing Interest

All Authors contributed to this work in the following ways: All authors have read and approved the final manuscript.

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