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# Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network



RESEARCH ARTICLE

BIOSCIENCE RESEARCH, 2020 17(3):1865-1870.

OPEN ACCESS

## Influence of Soaking Time and Incubation Temperature to Chemical, Phytochemical and Antinutritional Properties of Germinated Winged Bean (*Psophocarpus tetragonolobus*)

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Winged bean (*Psophocarpus tetragonolobus*) is a tropical underutilized legume with beneficial nutritional characteristics. Its seed has a huge amount of nutritional and phytochemical compounds with excellent antioxidant capacity. However, winged bean seed also contains harmful substances like tannin and phytate. These antinutrients cause malnutrition in consumption. The application of thermal treatment has been shown to safely eliminate these harmful elements without any degradation in its nutritional composition. Our research attempted to demonstrate the effectiveness of soaking time (8, 12, 16, 20, 24 hours) and incubation temperature (30, 32, 34, 36, 38, 40°C) in germination to vitamin C (mg/100g), vitamin E (mg/100g), total phenolic (mg GAE/100g), flavonoid (mg QE/100g), DDPH (%) radical scavenging assay, tannin (%), phytate (mm/100g) of the germinated winged bean. Our results revealed that the highest accumulations of vitamin C, vitamin E, total phenolic, flavonoid content as well as the maximum DPPH, tannin, phytate reduction were observed by soaking time 20 hours and incubation temperature 36°C. Through germination, the nutrients could be enhanced while antinutrient factors would be degraded significantly in the germinated winged bean.

**Keywords:** Winged bean, soaking, germination, incubation, nutrient, antinutrient

### INTRODUCTION

Winged bean (*Psophocarpus tetragonolobus*) is an underutilized legume widely distributed in tropical regions (Achal and Rajeshwar, 2018). It is a legume crop with a rich source of protein, carbohydrate and oil content (Mahto and Dua, 2009; Chandra et al., 2013; Singh et al., 2013; Prasanth et al., 2015). Amoo et al. (2006) reported that the proximate composition of winged bean seed had 9.22% moisture, 4.91% ash, 17.51% fat, 12.23% crude fibre, 33.83% crude protein and 22.30% carbohydrate. Moreover, winged bean also contains a huge amount of phenolic content with excellent antioxidant capacity (Mohd et al., 2013). With specific

nutritional quality, it provides food source at all stages of its life cycle (Mohammad et al. 2016). Winged bean can be consumed both as a vegetable and snack. The raw and processed winged bean flours could be used as functional ingredient in food preparation like bakery, whipped topping, weaning food, vegetable milk (Igene et al., 2005). It could be fermented by *Lactobacillus plantarum* into pickle product (Minh et al., 2019). However, winged bean also contains antinutrients like tannin and phytate. The presence of antinutrients (tannin and phytate) in winged bean could hinder the efficient utilization and digestion of functional nutrients (Taofeek et al., 2019). Tannin had cross-link with proteins and

caused a reduction protein digestion of beans (Jacob et al., 2015; Inyang et al., 2015; Ajibola et al., 2016). The inhibition of digestive enzymes increased excretion of endogenous protein affecting to digestive tract (Parmar et al., 2017). Phytate is an antinutrient factor normally existed in legume seeds. It is an antioxidant possibly binding to dietary minerals, interfering with their availability (Tan et al.1983).

Richard and Prapasri(1987) examined effects of germination on the proximate composition and nutritional quality of winged bean (*Psophocarpus tetragonolobus*) seeds. The nonprotein nitrogen was increased gradually while protein nitrogen decreased. A minor decrease in the lipid content was noticed. Trypsin inhibitor activity was unaffected by germination. Lipoxygenase activity was decreased dramatically. A decrease of the amino acid content was recorded after 48 hours of germination. Meanwhile a significant increase in cysteine, aspartic acid, and histidine content were found after 72 hours of incubation. Objective of our study focused on the the effectiveness of soaking time and incubation temperature in germination to nutritional accumulation and antinutritional degradation of the germinated winged bean.

## MATERIALS AND METHODS

### Material

Winged beans were collected from SocTrang province, Vietnam. After collecting, they were cleaned by tap water to remove foreign matters before soaking and germination. Chemicals and reagents such as ethanol, FolinCiocalteu reagent, sodium carbonate, gallic acid, rutin, iodine,  $\alpha$ , $\alpha$ -dipyridyl, ferric, 2, 2-diphenyl-1-picrylhydrazyl were all analytical grade and purchased from Merk or Sigma-Aldrich.

### Researching method

#### Effect of soaking time to chemical and phytochemical characteristics of soaked winged bean

Clean winged beans were soaked in water in different soaking times (8, 12, 16, 20, 24 hours) at ambient temperature. The soaked winged beans were drained and sampled to analyze vitamin C (mg/100g), vitamin E (mg/100g), total phenolic (mg GAE/100g), flavonoid (mg QE/100g), DPPH (%), tannin (%), phytate (mg/100g).

#### Effect of incubation temperature to chemical and phytochemical characteristics of germinated winged bean

The soaked winged bean would be then incubated at different temperature (30, 32, 34, 36, 38, 40°C) for 72 hours to facilitate germination. These sprouts from germination process would be sampled to evaluate vitamin C (mg/100g), vitamin E (mg/100g), total phenolic (mg GAE/100g), flavonoid (mg QE/100g), DPPH (%), tannin (%), phytate (mg/100g).

#### Chemical and phytochemical analysis

Vitamin C content (mg/100g) was determined by redox titration with iodine (Mussa and Sharaa, 2014). Vitamin E content (mg/100g) was determined by Emmerie Engel reaction (Rutkowski and Grzegorzczuk, 2007). Total phenolic content (mg GAE/g) was evaluated using Folin–Ciocalteu assay (NizarSirag et al., 2014). Total flavonoid content (mg QE/g) was evaluated by the aluminium calorimetric method (Formagio et al., 2015).The antioxidant activity was evaluated using DPPH (%) radical scavenging assay which was described by Huang et al. (2005). Tannin (%) was determined as analyzing assay described by Adegunwa et al. (2011). Phytate (mg/100g) was estimated as analyzing assay described by Wheeler and Ferrel (1971).

#### Statistical analysis

The experiments were run in triplicate with three different lots of samples. The data were presented as mean $\pm$ standard deviation. Statistical analysis was performed by the Statgraphics Centurion version XVI.

## RESULTS AND DISCUSSION

#### Effect of soaking time to vitamin C, vitamin E, total phenolic, flavonoid, DPPH, tannin, phytate of soaked winged bean (*Psophocarpus tetragonolobus*)

Result shown in Table 1 revealed that vitamin C, vitamin E, total phenolic, flavonoid, tannin, phytate in winged bean decreased significantly after soaking leading to increase in DPPH. The soaking time should be stopped at 20 hours ready for germination. This phenomenon could be explained by the leaching of water soluble components into soaking water. Our data were similar to other findings on various material such as seed legume (Xu and Chang, 2008), soybean (Duong et al., 2016), chickpea (Aharon et al., 2011), sorghum (Afify et al., 2012).

**Table 1: Effect of soaking time (hours) to vitamin C, vitamin E, total phenolic, flavonoid and DPPH of soaked winged bean (*Psophocarpus tetragonolobus*)**

Soaking time (hours)	Non soaking	8	12	16	20	24
Vitamin C (mg/100g)	246.19±0.01 <sup>a</sup>	231.54±0.00 <sup>ab</sup>	214.07±0.03 <sup>b</sup>	196.73±0.01 <sup>bc</sup>	174.11±0.03 <sup>c</sup>	173.62±0.00 <sup>c</sup>
Vitamin E (mg/100g)	5.12±0.03 <sup>a</sup>	5.04±0.01 <sup>ab</sup>	4.97±0.00 <sup>b</sup>	4.91±0.02 <sup>bc</sup>	4.83±0.01 <sup>c</sup>	4.81±0.02 <sup>c</sup>
Total phenolic (mg GAE/100 g)	293.07±0.00 <sup>a</sup>	284.10±0.02 <sup>ab</sup>	273.11±0.01 <sup>b</sup>	244.57±0.03 <sup>bc</sup>	230.49±0.00 <sup>c</sup>	229.65±0.01 <sup>c</sup>
Flavonoid (mg QE/100 g)	96.25±0.01 <sup>a</sup>	90.17±0.03 <sup>ab</sup>	83.46±0.00 <sup>b</sup>	74.85±0.01 <sup>bc</sup>	62.78±0.02 <sup>c</sup>	61.95±0.00 <sup>c</sup>
DPPH(%)	28.36±0.03 <sup>c</sup>	29.10±0.02 <sup>bc</sup>	29.95±0.03 <sup>b</sup>	30.78±0.00 <sup>ab</sup>	31.43±0.03 <sup>a</sup>	31.49±0.02 <sup>a</sup>
Tannin (%)	1.75±0.02 <sup>a</sup>	1.61±0.00 <sup>ab</sup>	1.53±0.03 <sup>b</sup>	1.40±0.00 <sup>bc</sup>	1.31±0.03 <sup>c</sup>	1.29±0.00 <sup>c</sup>
Phytate (mg/100g)	5.21±0.01 <sup>a</sup>	5.04±0.03 <sup>ab</sup>	4.83±0.02 <sup>b</sup>	4.71±0.01 <sup>bc</sup>	4.57±0.00 <sup>c</sup>	4.55±0.01 <sup>c</sup>

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ( $\alpha = 5\%$ ).

**Table 2: Effect of incubation temperature (°C) to vitamin C, vitamin E, total phenolic, flavonoid and DPPH of soaked winged bean (*Psophocarpus tetragonolobus*) after 72 hours of incubation**

Incubation (°C)	Before germination	30	32	34	36	38	40
Vitamin C (mg/100g)	174.11±0.03 <sup>e</sup>	215.31±0.01 <sup>d</sup>	269.74±0.02 <sup>c</sup>	334.82±0.01 <sup>b</sup>	397.56±0.00 <sup>a</sup>	346.10±0.00 <sup>ab</sup>	301.12±0.02 <sup>bc</sup>
Vitamin E (mg/100g)	4.83±0.01 <sup>d</sup>	4.97±0.00 <sup>cd</sup>	5.25±0.03 <sup>c</sup>	5.77±0.02 <sup>bc</sup>	6.43±0.01 <sup>a</sup>	6.16±0.02 <sup>ab</sup>	5.90±0.03 <sup>b</sup>
Total phenolic (mg GAE/100 g)	230.49±0.00 <sup>d</sup>	327.56±0.03 <sup>cd</sup>	394.63±0.01 <sup>c</sup>	477.54±0.03 <sup>bc</sup>	539.60±0.02 <sup>a</sup>	517.09±0.01 <sup>ab</sup>	492.34±0.00 <sup>b</sup>
Flavonoid (mg QE/100 g)	62.78±0.02 <sup>d</sup>	71.82±0.02 <sup>cd</sup>	86.79±0.00 <sup>c</sup>	98.57±0.01 <sup>bc</sup>	167.23±0.03 <sup>a</sup>	134.07±0.01 <sup>ab</sup>	116.71±0.03 <sup>b</sup>
DPPH (%)	31.43±0.03 <sup>a</sup>	30.79±0.01 <sup>ab</sup>	28.58±0.03 <sup>b</sup>	26.02±0.02 <sup>bc</sup>	22.64±0.01 <sup>d</sup>	23.96±0.02 <sup>cd</sup>	24.84±0.01 <sup>c</sup>
Tannin (%)	1.31±0.03 <sup>a</sup>	1.15±0.02 <sup>ab</sup>	1.0±0.02 <sup>bc</sup>	0.90±0.02 <sup>cd</sup>	0.78±0.02 <sup>d</sup>	0.98±0.02 <sup>c</sup>	1.09±0.02 <sup>b</sup>
Phytate (mg/100g)	4.57±0.00 <sup>a</sup>	4.23±0.01 <sup>ab</sup>	4.09±0.01 <sup>b</sup>	3.72±0.01 <sup>cd</sup>	3.56±0.01 <sup>d</sup>	3.83±0.01 <sup>c</sup>	3.95±0.01 <sup>bc</sup>

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ( $\alpha = 5\%$ )

**Effect of incubation temperature to vitamin C, vitamin E, total phenolic, flavonoid, DPPH, tannin, phytate of germinated winged bean (*Psophocarpus tetragonolobus*)**

Result shown in table 2 revealed that vitamin C, vitamin E, total phenolic, flavonoid in winged bean increased significantly after incubation from 30°C to 36°C leading to decrease in DPPH. Tannin and phytate decreased dramatically at incubation from 30°C to 36°C. However at higher temperature (38-40°C), these chemical and phytochemical contents decreased gradually with an exception of tannin and phytate. The incubation temperature should be conducted at 36°C to obtain the highest functional constituents. The increasing of vitamin C, vitamin E, total phenolic, flavonoid and vitamin C in winged bean at 36°C of incubation could be explained via the biosynthesis and bioaccumulation of

phytochemical constituents as well as reactivation of enzyme (L-Galactono- $\gamma$ -lactone dehydrogenase) involved in the oxidation of L-galactono-1, 4-lactone to ascorbic acid during germination (Xu et al., 2005). Our data were in agreement with other findings on various material such as soybean (Ahmad and Pathak, 2000; Vasantharuba et al., 2007; Zhou and Zhang, 2012; Duong et al., 2016; Kou and Zhou, 2016), edible seed (Cevallos et al., 2010), lentil (Fouad and Rehab, 2015), mung bean (Randhir et al., 2004), cow pea (Doblado et al., 2007), chickpea (Masood et al., 2014), legume (Davey et al., 2000), sorghum (Suryanti et al., 2016).

**CONCLUSION**

Winged bean possesses free phenolics, tannins, phytic acid, flatulence factors, saponins, and hydrogen cyanide. In this research, we have successfully found that soaking and germination had safely eliminated antinutritive factors while

enhancing nutritional and phytochemical constituents. From this finding, the added value of winged bean would be enhanced significantly. Consumer have more chance to use this functional product for healthy benefits.

### CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

### ACKNOWLEDGEMENT

We acknowledge the financial support for the publication provided by Thu Dau Mot University, Vietnam.

### AUTHOR CONTRIBUTIONS

Nguyen Phuoc Minh arranged the experiments and also wrote the manuscript.

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