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Technical variables influencing the seed germination of winged bean (*Psophocarpus tetragonolobus*) seed

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Abstract

The demand for sprouted seeds as dietetics and exotic healthy foods has recently increased. Winged bean seeds contain both nutritional composition and antinutrients. In order to convert this seed into healthy food, the winged bean was germinated into a vegetable sprout. In this research, the influence of soaking and sprouting conditions on the physicochemical and sensory attributes of sprouts was observed. Results showed that winged bean should be soaked in water at 34°C in 7 hrs, water to solid 3/1. The soaked seed was drained for 15 mins and incubated at temperature 36°C for 18 hrs. Following these parameters, the winged bean sprout achieved extended length (46.25±0.02 mm), vitamin C (124.13±0.01 mg/100 g), total phenolic content (997.34±1.14 mg GAE/100 g) and overall acceptance (8.94±0.02). Soaking terms for sprouting could improve the nutritional attribute of the winged bean as a functional foodstuff.

1. Introduction

Sprouting is a simple and cheap method to provide healthy foodstuffs with high yield, without complicated utensils, in a short production cycle in a small warehouse (Delian *et al.*, 2015; Kyriacou *et al.*, 2016; Paolo *et al.*, 2019). Germination begins with water absorption and terminates with the extension of the embryo axis. Physical and biochemical changes occur during this process (Logan *et al.*, 2001). Sprouting induces a mild accumulation of the total polyphenolic content (Alvarez-Jubete *et al.*, 2010; Pal *et al.,* 2016). The ascorbic acid content in legumes is quite low, those accumulated greatly in sprouted barley (Danisova *et al.*, 1994) and wheat (Yang *et al.*, 2001; Plaza *et al.*, 2003) is due to its de novo synthesis (Lemmens *et al.*, 2018). Germination induces the accumulation of total phenolic content in lupin seeds (Duenas *et al.*, 2009). The ascorbic acid content in mung beans increases during germination (Shah *et al.*, 2011) and directly alters the total phenolic content greatly affecting the antioxidant capacity of sprouts (Lopez-Amoros *et al.*, 2006). In germination, the cell wall degrades to release free phenolics (Gujral *et al.*, 2011). Modification of total phenolic content was also noticed on sorghum sprout (Sorour *et al.*, 2017). Total phenolic content was degraded dramatically in germinated peanut and soybeans due to enzymatic reactions (Megat and Azrina, 2012). In rice sprouting, a high amount of antioxidants, bioactive substances and antioxidant activity were accumulated (Maninder *et al.*, 2017).

Winged bean (*Psophocarpus tetragonolobus*) seed is an underutilized legume with beneficial nutritional composition (protein, fat, carbohydrate, mineral) and antinutrient (tannin and phytate) (Taofeek *et al.*, 2019). Winged bean seed is highly appreciated as compared to soybean in respect of its excellent nutritional value and high yield but limited to the amino acid cysteine and methionine (Novelina *et al.*, 2013; Vatanparast *et al.*, 2016). Their sprout can be processed into a drink (Novelina *et al.*, 2013) despite its unpleasant flavour due to the activity of lipoxygenase. Sprouting is an effective approach to overcome the rotten feeling from this bean. Lipoxygenase activity was eliminated 77% of the original activity after 120 hrs of germination (Richard and Prapasri, 2006). The main objective of our study was to observe the effect of soaking and sprouting conditions on the sprout length, total phenolic content, vitamin C and sensory attribute of sprouts.

2. Materials and methods

2.1 Materials

Winged bean seed was collected from the gardens in Soc Trang province, Vietnam. It was dried under

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sunlight to 14% moisture content and kept in a cool place. Chemical reagents were all analytical grade (> 99% in purity) purchased from Rainbow Trading Co. Ltd., Vietnam.

2.2 Experiments

Experiment 1: Effect of soaking temperature on physicochemical and sensory properties of sprouts. The seed was soaked in water at different temperatures (30, 32, 34, 36, 38°C) within 4 hrs, with a water to solid ratio of 1/1. The soaked seed was drained for 15 mins before incubation at 30°C for 6 hrs. The sprout was then collected for physicochemical and sensory evaluation to define the optimal soaking temperature.

Experiment 2: Effect of soaking time on physicochemical and sensory properties of sprouts. The seed was soaked in water at 34°C in different durations (4, 5, 6, 7, 8 hrs), water to solid ratio 1/1. The soaked seed was drained 15 mins before incubation at 30°C for 6 hrs. The sprout was collected for physicochemical and sensory evaluation to define the optimal soaking time.

Experiment 3: Effect of water to seed ratio during soaking to physicochemical and sensory properties of sprout. The seed was soaked in water at 34°C for 7 hrs, with different ratios of water to solid (1/1, 2/1, 3/1, 4/1, 5/1). The soaked seed was drained for 15 mins before incubating at 30°C for 6 hrs. The sprouts were collected ready for physicochemical and sensory evaluation to define the optimal water to seed ratio.

Experiment 4: Effect of sprouting temperature on physicochemical and sensory properties of sprouts. The seed was soaked in water at 34°C for 7 hrs, water to solid 3/1. The soaked seed was drained for 15 mins. Incubation was conducted different temperatures (30, 32, 34, 36, 38°C) for 6 hrs. The sprout was collected for physicochemical and sensory evaluation to define optimal sprouting temperature.

Experiment 5: Effect of sprouting time on physicochemical and sensory properties of sprouts. The seed was soaked in water at 34°C for 7 hrs, water to solid 3/1. The soaked seed was drained for 15 mins. Incubation was conducted at temperature 36°C in different durations (6, 12, 18, 24, 30 hours). The sprouts were then collected for physicochemical and sensory evaluation to define optimal sprouting time.

The target function in each experiment was based on the length of sprout (mm), vitamin C (mg/100 g) and overall acceptance.

2.3 Physicochemical evaluation

Length of sprout (mm) was measured by millimetre tape. Vitamin C content (mg/100 g) was quantified according to the method described by AOAC (2005). Total phenolic content (mg GAE/100 g) was estimated by Folin-Ciocalteu reagent assay (Singleton and Rossi, 1965). Overall acceptance was evaluated by a group of panellists using a 9-point Hedonic scale.

2.4 Statistical analysis

The demonstrations were prepared as three replicates for various sample groups. The values were expressed as mean ± standard deviation. The statistical analysis was done using Statgraphics version XVI.

3. Results and discussion

3.1 Effect of soaking temperature to physicochemical and sensory properties of sprout

Soaking temperature significantly affected the physicochemical and sensory attributes of sprouts (Table 1). When the soaking temperature increased in the range of 30-34°C, the sprout length extended from 24.71±0.03 to 29.14±0.03 mm. When the soaking temperature surpassed 34°C, the sprout length slightly decreased. Similarly, other parameters of vitamin C (39.46±0.01 to 62.27±0.01 mg/100 g), total phenolic content (187.40±1.21 to 349.06±1.28 mg GAE/100 g) and overall acceptance (4.19±0.01 to 6.13±0.02) was also noticed when soaking temperature increased in the range of 30-34°C. 34°C was the optimal soaking temperature. In soaking, different complicated components were broken down into simpler substances by biological reactions (Narsih and Harijono, 2012). The 6 hrs soaking induced the extension of the pistachio shoot (Esmaeilpour and Damme, 2016). When soaking at 33°C, the total phenolic content in germinated brown rice was noticed at the highest level of 33.95 mg/100 g (Kanjana *et al.*, 2017).

3.2 Influence of soaking time on physicochemical and sensory properties of sprout

The effect of soaking time on physicochemical and sensory attributes of sprouts was expressed in Table 2. Sprout length, vitamin C, total phenolic content, overall acceptance increased dramatically when the soaking time extended to 7 hrs. There was no significant difference between 7 and 8 hrs in respect of mentioned parameters. Within 7 hrs of soaking, sprout length (34.08±0.03 mm), vitamin C (76.49±0.01 mg/100 g), total phenolic content (587.34±1.09 mg GAE/100 g), overall acceptance (7.25±0.04) were recorded. Soaking time in 7 hrs was selected for the next experiments. After soaking for 300 mins, the total phenolic content in germinated brown rice was observed at the highest level at 33.95 mg/100 g (Kanjana *et al.*, 2017). Steeping for 24 hrs at 31°C was suitable for sorghum germination (Sharma *et al.*, 2015).

Table 1. Effect of soaking temperature to physicochemical and sensory properties of sprout

Soaking temperature (^{o}C)	30	32	34	36	38
Sprout length (mm)	24.71±0.03^c	25.39±0.02^{bc}	29.14±0.03^a	28.09±0.01^{ab}	26.85±0.02^b
Vitamin C (mg/100 g)	39.46±0.01^c	45.15±0.03^{bc}	62.27±0.01^a	56.33±0.00^{ab}	50.28±0.03^b
Total phenolic (mg GAE/100 g)	187.40±1.21^c	246.19±1.34^{bc}	349.06±1.28^a	301.25±1.04^{ab}	274.31±1.15^b
Overall acceptance	4.19±0.01^c	4.57±0.03^{bc}	6.13±0.02^a	5.61±0.01^{ab}	5.02±0.03^b

Values are presented as mean±SD, n = 3. Values with the same superscript within the same row are not significantly different (α = 5%).

Table 2. Effect of soaking time to physicochemical and sensory properties of sprout

Soaking time (hours)	4	5	6	7	8
Sprout length (mm)	29.14±0.03^c	30.08±0.00^{bc}	31.25±0.01^b	34.08±0.03^a	34.15±0.02^a
Vitamin C (mg/100 g)	62.27±0.01^c	65.47±0.02^{bc}	69.23±0.00^b	76.49±0.01^a	76.83±0.03^a
Total phenolic (mg GAE/100 g)	349.06±1.28^c	396.10±1.46^{bc}	452.09±1.28^b	587.34±1.09^a	590.21±1.32^a
Overall acceptance	6.13±0.02^c	6.45±0.00^{bc}	6.83±0.03^b	7.25±0.04^a	7.09±0.02^{ab}

Values are presented as mean±SD, n = 3. Values with the same superscript within the same row are not significantly different (α = 5%).

Foxtail millet accumulated the highest total phenolic content after soaking in portable water at ambient temperature (Sharma *et al.*, 2015). During 24 hrs of soaking, there was an accumulation of total phenolics in brown rice (Bishnoi *et al.*, 1994) and wheat (Yang *et al.*, 2001).

3.3 Impact of water to seed ratio during soaking to physicochemical and sensory properties of sprout

Effect of water/seed ratio to physicochemical and sensory attributes of sprout was expressed in Table 3. The more water incorporated (1/1 to 4/1), the longer sprout length was recorded (34.08±0.03 to 38.50±0.07 mm). However, vitamin C (76.49±0.01 to 87.14±0.01 mg/100 g), total phenolic content (587.34±1.09 to 736.54±1.14 mg GAE/100 g), and overall acceptance (7.25±0.04 to 8.01±0.00) accumulated significantly when the water/seed ratio increased from 1/1 to 3/1. When water was added to the seeds at 4/1 or 5/1, vitamin C (85.16±0.03 to 83.46±0.02 mm), total phenolic content (694.16±1.18 mg GAE/100 g), and overall acceptance (7.83±0.01 to 7.72±0.03) gradually decreased. Therefore, the water/seed ratio was fixed at 3/1 for the next experiments. Moisture penetrates the seeds through the membrane by diffusion as a result of the accumulation of endogenous osmotica (Obroucheva and Antipova, 1997; Obroucheva *et al.*, 2017). Moisture inflow into cells happened through water channels made by plasmalemma aquaporins (Trofimova *et al.*, 2001; Javot and Maurel, 2002). The proteolysis of reserve proteins initiated by proteinases if the moisture content in embryonic axes reached about 45–55% (Muntz *et al.*, 2001). At moisture of 55–65%, the degradation of legumin induced by endopeptidases originated from the cytoplasm (Lichtenfeld *et al.*, 1979). At moisture of 68%, vacuoles were rather visible and enlarged, ready for further absorption.

3.4 Effect of sprouting temperature to physicochemical and sensory properties of sprout

The influence of sprouting temperature on sprout length, vitamin C, total phenolic content, overall acceptance was presented in Table 4. Sprout length (36.85±0.02 to 41.25±0.03 mm), vitamin C (87.14±0.01 to 96.14±0.02 mg/100 g), total phenolic content (736.54±1.14 to 897.47±1.09 mg GAE/100 g), and overall acceptance (8.01±0.00 to 8.64±0.02) greatly improved when the sprouting temperature increased from 30 to 36^{o}C. At 38^{o}C, those aforementioned parameters decreased gradually. Hence, optimal sprouting temperature was chosen at 36^{o}C for the next experiment. 30^{o}C was optimal for maximum sprouting, stem length and yield from mungbean seed (Muhammad *et al.*, 2019). A temperature of 35^{o}C was ideal for sprouting

Table 3. Effect of water to seed ratio during soaking to physicochemical and sensory properties of sprout

Water to seed ratio	1/1	2/1	3/1	4/1	5/1
Sprout length (mm)	34.08±0.03^c	35.46±0.05^{bc}	36.85±0.02^b	37.69±0.02^{ab}	38.50±0.07^a
Vitamin C (mg/100 g)	76.49±0.01^c	81.23±0.03^{bc}	87.14±0.01^a	85.16±0.03^{ab}	83.46±0.02^b
Total phenolic (mg GAE/100 g)	587.34±1.09^c	628.14±1.37^{bc}	736.54±1.14^a	694.16±1.18^{ab}	662.08±1.05^b
Overall acceptance	7.25±0.04^c	7.54±0.03^{bc}	8.01±0.00^a	7.83±0.01^{ab}	7.72±0.03^b

Values are presented as mean±SD, n = 3. Values with the same superscript within the same row are not significantly different (α = 5%).

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Table 4. Effect of sprouting temperature to physicochemical and sensory properties of sprout

Sprouting temperature (°C)	30	32	34	36	38
Sprout length (mm)	36.85±0.02^{c}	37.62±0.03^{bc}	38.33±0.01^{b}	41.25±0.03^{a}	40.07±0.04^{ab}
Vitamin C (mg/100 g)	87.14±0.01^{c}	89.40±0.00^{bc}	91.63±0.03^{b}	96.14±0.02^{a}	93.84±0.00^{ab}
Total phenolic (mg GAE/100 g)	736.54±1.14^{c}	772.46±1.11^{bc}	811.24±1.06^{b}	897.47±1.09^{a}	864.08±1.01^{ab}
Overall acceptance	8.01±0.00^{c}	8.17±0.02^{bc}	8.35±0.03^{b}	8.64±0.02^{a}	8.50±0.01^{ab}

Values are presented as mean±SD, n = 3. Values with the same superscript within the same row are not significantly different (α = 5%).

black gram (Danaipot *et al.* 2015). Sorghum should be sprouted within 4.5 days at 30°C to obtain the highest total phenolic content (Sharma *et al.*, 2015). The highest total phenolic content was obtained in the germination of grain at 28°C for 96 hrs (Caceres *et al.*, 2014). The highest total phenolic content in wheat sprouts was obtained after 7 days of germination at 16.5°C (Yang *et al.*, 2001). The optimal temperature for rambutan sprouting was 25°C in six days (Renata *et al.*, 2017).

3.5 Effect of sprouting time to physicochemical and sensory properties of sprout

Effect of sprouting time to sprout length, vitamin C, total phenolic content and overall acceptance was elaborated in Table 5. The longer the sprouting time was extended (6-30), the longer the length of the sprout (41.25±0.03 to 52.64±0.02 mm). However, vitamin C (124.13±0.01 mg/100 g), total phenolic content (997.34±1.14 mg GAE/100 g) and overall acceptance (8.94±0.02) could be obtained at 18 hrs of sprouting. Soaking cashew nuts in water for 24 or 36 hrs in advance of sowing resulted in high cashew crop (Oyewole and Koffa, 2010). In waxy wheat, the total phenolic content dropped after 12 and 24 hrs of sprouting and then greatly accelerated after 36 and 48 hrs (Hung *et al.*, 2012). In brown rice, both free- and bound- fragments greatly increased over the sprouting period owing to the hydrolysis of conjugated phenolic components (Ti *et al.*, 2014; Zilic *et al.*, 2014). Foxtail millet accumulated the highest total phenolic content after 40 hrs of sprouting at 25°C (Sharma *et al.*, 2015). Purple corn sprouts had the highest total phenolic content by sprouting at 26°C for 63 hrs (Paucar-Menacho *et al.*, 2017). Sprouted quinoa received much more phenolics by germination at 20°C for 42 hrs (Paucar-Menacho *et al.*, 2018). The antioxidant activity of germinated seeds was affected by sprouting duration (Suryanti *et al.*, 2016).

4. Conclusion

The Winged bean is an underexploited tropical leguminous plant. Its seed attracts great commercial interest mainly due to its proximate composition. It can be considered as an alternative for soybean with similar characteristics. Successfully observed changes of sprout length, total phenolic content, vitamin C and organoleptic attribute under different conditions of soaking (time, temperature, water to solid ratio) and germination (time and temperature) were recorded. Sprouts from winged beans would be as beneficial for human health as green vegetables.

Conflict of interest

The author strongly confirms that this research was conducted with no conflict of interest.

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Table 5. Effect of sprouting time to physicochemical and sensory properties of sprout

Soaking time (hours)	6	12	18	24	30
Sprout length (mm)	41.25±0.03^{c}	43.59±0.01^{bc}	46.25±0.02^{b}	49.37±0.00^{ab}	52.64±0.02^{a}
Vitamin C (mg/100 g)	96.14±0.02^{c}	105.37±0.03^{bc}	124.13±0.01^{a}	115.28±0.03^{ab}	108.30±0.01^{b}
Total phenolic (mg GAE/100 g)	897.47±1.09^{c}	925.68±0.85^{bc}	997.34±1.14^{a}	980.41±1.04^{ab}	957.12±1.25^{b}
Overall acceptance	8.64±0.02^{c}	8.71±0.00^{bc}	8.94±0.02^{a}	8.86±0.01^{ab}	8.80±0.00^{b}

Values are presented as mean±SD, n = 3. Values with the same superscript within the same row are not significantly different (α = 5%).

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