Original Article

Content and Stability of Total Phenolic Compounds and Antioxidant Capacity of Matured Shallot Bulbs' Extract (*Allium ascalonicum* L.)

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Background: *Due to restrictions of nutritional data and researches on shallots of Srisaket variety (Allium ascalonicum L.), their value-added applications have been found to be limited.* ***Objectives:*** *To examine content and stability of total phenolic compounds and antioxidant capacity of matured shallot Bulbs (Srisaket variety).* ***Materials and Methods:*** *Matured bulb samples were processed to be extracted by using aqueous extraction, prior to analysis for total phenolic compounds and antioxidant capacity. For stability study in native bulbs, both parameters were assessed monthly.* ***Results:*** *From 21 samples analyzed, the highest values of total phenolic compounds of 369.54-406.92 mg/g dried weight, were found in the peel part, comparing to the values of 190.56-221.34 mg/g dried weight of the whole bulbs, and 115.13-128.93 mg/g dried weight of the inner part. In the case of antioxidant capacity, levels of 0.130-0.162, 0.113-0.128, and 0.007-0.009 mg/g dried weight were assayed in the peel part, whole bulbs and the inner part, respectively. In term of stability, no significant changes of both parameters were observed in any parts of bulb samples, when stored at room temperature conditions for three months.* ***Conclusions:*** *The highest levels of total phenolic compounds and antioxidant capacity were found in the peel part of matured shallot bulbs of Srisaket variety, and their stability of native bulbs were not changed for at least three months.*

Keywords: ● *Shallots of Srisaket variety* ● *Antioxidant capacity* ● *Total phenolic compounds* ● *Stability*

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นิพนธ์ต้นฉบับ

ปริมาณ และเสถียรภาพ ของสารประกอบฟีนอลิกทั้งหมดและฤทธิ์ต้านอนุมูลอิสระของสารสกัดจากหัวหอมแดงสายพันธุ์ศรีสะเกษ

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บทคัดย่อ

ความเป็นมา *เนื่องจากงานวิจัยและข้อมูลทางโภชนาการของหอมแดงสายพันธุ์ศรีสะเกษมีอย่างจำกัด จึงส่งผลต่อการสร้างมูลค่าเพิ่มให้กับหอมแดง* ***วัตถุประสงค์*** *เพื่อศึกษาหาปริมาณและเสถียรภาพของสารประกอบฟีนอลิกทั้งหมดและฤทธิ์ต้านอนุมูลอิสระของสารสกัดจากหอมแดงสายพันธุ์ศรีสะเกษ* ***วิธีการศึกษา*** *ตัวอย่างหอมแดงสายพันธุ์ศรีสะเกษที่เจริญเต็มที่ ถูกนำมาสกัดด้วยวิธีการสกัดด้วยน้ำ จากนั้นทำการวิเคราะห์หาค่าสารประกอบฟีนอลิกทั้งหมด และค่าฤทธิ์ต้านอนุมูลอิสระในสารสกัดดังกล่าว และทำการศึกษาเสถียรภาพโดยการวิเคราะห์หาค่าสารทั้งสองชนิดนี้ในตัวอย่างหอมแดงทุกๆเดือน* ***ผลการศึกษา*** *จากการวิเคราะห์ตัวอย่างหอมแดงที่เจริญเต็มที่จำนวน 21 ตัวอย่าง พบว่า ค่าสารประกอบฟีนอลิกทั้งหมดจากสารสกัดส่วนเปลือกนอก มีค่าสูงสุดเท่ากับ 369.54-406.92 มิลลิกรัมต่อกรัมน้ำหนักแห้งสกัดแห้ง เทียบกับค่าที่พบในสารสกัดของส่วนทั้งหัวและส่วนชั้นใน เท่ากับ 190.56-221.34 และ 115.13-128.93 มิลลิกรัมต่อกรัมน้ำหนักแห้ง ตามลำดับ กรณีของค่าฤทธิ์ต้านอนุมูลอิสระ พบว่าสารสกัดจากส่วนเปลือกนอก ส่วนทั้งหัว และส่วนชั้นใน มีค่าเท่ากับ 0.130-0.162, 0.113-0.128, และ 0.007-0.009 มิลลิกรัมต่อกรัมน้ำหนักแห้ง ตามลำดับ เมื่อทำการเก็บตัวอย่างหอมแดงไว้ที่สภาวะอุณหภูมิห้องเป็นระยะเวลาสามเดือน พบว่า ค่าสารประกอบฟีนอลิกทั้งหมดและค่าฤทธิ์ต้านอนุมูลอิสระที่วิเคราะห์ในสารสกัดจากตัวอย่างหอมแดง ไม่มีการเปลี่ยนแปลงอย่างมีนัยสำคัญ* ***สรุป*** *สารประกอบฟีนอลิกทั้งหมดและฤทธิ์ต้านอนุมูลอิสระพบว่ามีค่าสูงสุดในส่วนเปลือกนอกของหัวหอมแดงสายพันธุ์ศรีสะเกษ และคงอยู่ได้นานอย่างน้อย 3 เดือน*

คำสำคัญ: ● *หอมแดงสายพันธุ์ศรีสะเกษ* ● *สารประกอบฟีนอลิกทั้งหมด* ● *ฤทธิ์ต้านอนุมูลอิสระ* ● *เสถียรภาพ*
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Background

In order to protect human from various diseases, attempts to discover new phytochemicals with their promising capacities to inhibit free radical, have been increasingly interested as an important sector of current research trends[1-2]. Allium Plants such as onions, garlics and shallots, were insisted for dietetic benefits to human, due to their content of potential condiments with a wide range of nutraceutical capacities e.g. anti-inflammation, anti-mutagenic, anti-cancer, anti-diabetic, anti-hypertensive, anti-biotic, and anti-oxidizing properties[3-10]. Shallots (*Allium ascalonicum* L.) have long been implied as one of popular spices among oriental food menus. Active ingredients analyzed from shallot bulb's extract e.g. flavone and polyphenolic derivatives such as quercetin, quercetin-4′-glucoside, quercetin-3,4′-diglucoside and quercetin mono-D-glucoside, were suggested to play the role in antioxidant capability and free radical scavenging property[11]. Interestingly, availability of free radical scavenging activity assayed in extracted samples from yellow and red onions, were found to be enriched highest at the outer layers, whereby continuous decreasing tendency was found towards the inner layers[12-13]. Contents of total phenolic compounds in plants had been reported to be varied from plant genetics, cultivars, soil quality, growing conditions, maturity state and harvest conditions[14].

Shallots of Srisaket variety, have been widely recognized as one of the top shallot varieties grown and distributed in Thailand[15-16]. Due to restrictions of nutritional data and food processing researches, their value-added applications have still found to be limited, leading to oversupplying problem annually[17-18]. Objectives of this study were: 1) to analyze content of total phenolic compounds and antioxidant capacity, and 2) to evaluate time-course stability of total phenolic compounds and antioxidant capacity in matured shallot bulb samples of Srisaket variety (*Allium ascalonicum* L.), grown in orchards of Srisaket province, Thailand.

Materials and Methods

Sample collection

Matured shallot bulbs of Srisaket variety (*Allium ascalonicum* L.) as air-dried frowns (0.5 kg per frown), were sampled from orchards in Srisaket province, Thailand. In order to obtain samples with relatively equal moisture content, all samples collected, were notably assured for at least 4 weeks, and not exceeded 6 weeks after harvesting.

Extraction & Optimal Extracting Condition

Shallot bulbs with horizontal diameters between 1.0 and 1.5 cm. and without sign of microbial growth, were randomly picked up to use in the experiments. After cleaning and knot-cutting, the peel (outer) part was taken by separating the two outer layers of each bulb sample, whereas the rest materials obtained later were assigned as the inner part. Either parts of shallot materials, as well as the whole bulb samples were dried at 100°C (hot air oven) until constant weights were obtained. All dried sample materials were processed to be grinded by using mortar. Optimal extracting condition carried out in aqueous solution, had been achieved as time and temperature algorithm. Briefly, 1 g of dried powder material was suspended in distilled water (50 mL) with different designed temperature (60 and 100°C). After shaking for 1 minute, all suspensions were maintained in a water bath (60 and 100°C) for 30, 60, and 120 minutes. Shaking (30 seconds each) was repeated every 30-minutes. Supernatants obtained after centrifuging (3,000 g, 15 min), were collected and preserved at -20°C, prior to analysis. Optimal extracting condition obtained from this study had been used throughout the experiments.

Analysis

Total phenolic compounds were assayed by Folin-Ciocalteu method using gallic acid as a standard[19]. Briefly, a 40-μL volume of supernatant sample was added into a 3.56-mL volume of distilled water. After mixing, a 100-μL volume of Folin-Ciocalteu reagent was added and then shaken. After incubating for 5 minutes, a 300-μL volume of 7% (w/v) fresh Na_2CO_3 solution was added, mixed, and maintained in a dark room for 2 hours. Absorbance value at 756 nm was read. Concentration of total phenolic compounds was measured from the calibration curve of gallic acid, and expressed as mg gallic acid equivalent per g dried weight sample.

In case of antioxidant activity, the measurement was done by Trolox equivalent antioxidant capacity method[20]. A 20-μL volume of supernatant sample was added into a 2-mL volume of ABTS solution containing in 10 mM phosphate buffer saline, pH 7.4 (2,2′-azino-bis (3-ethylbenzthiazoline 6-sulphonic acid, adjusted its absorbance values at 734 nm to be 7.00 ± 0.02). After mixing and incubating at ambient temperature for 1 minute exactly, the absorbance was read at 734 nm. Value of antioxidant capacity was measured from the calibration curve of Trolox, and expressed as mg Trolox equivalent per g dried weight sample. Each analysis was carried out in triplicates.

Time-course stability

To evaluate time-course stability of total phenolic compounds and antioxidant capability comprised in native samples, a batch of shallot bulbs as air-dried frowns (Srisaket variety, 0.5 kg per frown) taken at the same orchard, were maintained at ambient conditions. Briefly, frowns of shallot bulbs were hung up as top-down alignment, equipped with stretching wires, according to villagers' preserving method. Percentages of decayed or deformed bulbs were observed. Bulb samples with apparently good condition, were randomly selected to process for the measurement of total phenolic compounds and antioxidant activity as the procedure and methods prescribed. Experiments were carried out monthly over a three-month period.

Results

In order to imply for further large-scale and solvent-free preparations, extracting procedure used in this study were designed simply by using aqueous solution. Extraction at 60°C, yields of total phenolic compounds extracted from dried materials of whole bulb shallot samples, were increased periodically as extracting times progressed from 30 to 60 and 120 minutes (Table 1). When the extraction operated at 100 °C, the yields obtained at extracting times of 30 minutes (172.22-201.50 mg/mg dried weight) were approximately 90% of the yields achieved at 60 (190.10-224.84 mg/mg dried weight) and 120 minutes (188.40-228.70 mg/mg dried weight). Comparing the yields obtained at extracting times between 60 and 120 minutes that had been operated at 100 °C, they were not rather different (Table 1). Enhancing of extracting temperature from 60 to 100°C, yields of total phenolic compounds were observed

Table 1 Effect of temperature and extracting time on the amounts of total phenolic compounds (mg/g dried weight) extracted from dried powder of whole bulb shallot samples (Srisaket variety).

Extracting time (minutes)	Extracting temperature (°C)	
	60	100
30	80.50 - 110.34	172.22 - 201.50
60	120.84 - 145.80	190.10 - 224.84
120	172.23 - 205.63	188.40 - 228.70

increasingly in all extracting times used (30, 60 and 120 minutes). In conclusion, condition of using hot water (100 °C) and extracting time of 60 minutes, were optimally conducted for extracting phenolic compounds from dried material of shallot bulb samples, and used throughout the experiments.

From 21 samples analyzed, values of total phenolic compounds of 190.56 - 221.34, 369.54 - 406.92 and 115.13 - 128.93 mg/g dried weight, were contained in the whole bulbs, peel and inner parts of matured shallot bulb samples, respectively (Table 2). In a case of antioxidant capacity, values of 0.130 - 0.162 mg/g dried weight were shown to be existed of the peel part, comparing to 0.007 - 0.009 mg/g dried weight of the inner part, and 0.113 - 0.128 mg/g dried weight of the whole bulb samples (Table 2). Levels of total phenolic compounds comprised of the peel part were approximate 4 times higher than such content of the inner part, interestingly, levels of antioxidant capability of the peel part were approximately 10 times greater than the inner part's levels.

To evaluate stability of total phenolic compounds and antioxidant capacity in native samples, 12 frowns of shallot bulk samples were allowed to stay at ambient conditions with room temperature ranged between 19 and 35 °C, and relative humidity between 20 and 85%. Bulb weights were decreased continuously as storing time progressed, and the highest weight loss of 8.55% as observed at the first month of storing course (Table 3). At the end of three-month period, 3.76% of decayed bulks were observed, and almost bulbs appeared to be healthy with no sign of microbial growth (Table 3).

Antioxidant capacity and total phenolic compounds in the whole bulbs, inner and peel parts of shallot bulb samples, as analyzed monthly for a three-month period, was shown in Table 3. Tendencies for the content of total phenolic compounds of the whole bulbs and the peel part, and antioxidant capacity of the peel part, were presumably to be higher. By contrast, total phenolic compounds and antioxidant capacity of the inner part were a declined trend. When compared antioxidant

Table 2 Total phenolic compounds and antioxidant capacity analyzed in whole bulbs, peel and inner parts of 21 matured shallot bulb samples (Srisaket variety).

Shallot bulk sample analyzed	Levels of total phenolic compounds (mg/g dried weight)	Levels of antioxidant capability (mg/g dried weight)
Whole bulb	190.56 - 221.34	0.113 - 0.128
Peel part	369.54 - 406.92	0.130 - 0.162
Inner part	115.13 - 128.93	0.007 - 0.009

Table 3 Stability of total phenolic compounds and antioxidant capacity contained in native bulb samples of shallots (Srisaket variety) as stored at room temperature conditions for a three-month period.

Storing Duration (months)	Weight change per frown (%)	Average decayed bulbs (%)	Antioxidant capacity (mg/g dried weight)			Total phenolic compounds (mg/g dried weight)		
			Whole bulbs	Peel part	Inner part	Whole bulbs	Peel part	Inner part
0	0.00	0.00	0.120 ± 0.010	0.146 ± 0.018	0.008 ± 0.003	205.90 ± 16.60	398.20 ± 24.04	122.15 ± 9.55
1	-8.55	1.26	0.122 ± 0.010	0.143 ± 0.018	0.009 ± 0.003	200.55 ± 18.82	410.14 ± 28.80	118.88 ± 9.40
2	-12.43	2.80	0.118 ± 0.011	0.150 ± 0.018	0.008 ± 0.003	212.23 ± 18.60	418.64 ± 30.30	128.05 ± 10.15
3	-16.61	3.76	0.128 ± 0.012	0.155 ± 0.020	0.007 ± 0.003	218.00 ± 18.25	430.14 ± 34.22	111.32 ± 11.20

capacity and total phenolic compounds contained of the whole bulbs, inner and outer parts of shallot samples, between the 0-month and 3-month storing period, no significant change was demonstrated (paired t-test, $p < 0.001$)

Discussion

In this study, the extracting condition of using hot water (100°C) and extracting time of 60 minutes, were optimally conducted to extract phenolic compounds from powder materials of shallot bulbs. It was therefore, antioxidant activities contained in any extracts were insisted, depending on type and polarity of extracting solvents, isolation procedure, purity of active compounds and assayed techniques[21]. Extracting with less polar solvents, had been established to have higher yields of antioxidant activity than did with more polar solvent[22]. In this study, it should be noted that unpredictable amounts of phenolic compounds might be degraded during drying process in hot-air oven and/or hot-water extraction. Thermal treatment was revealed to affect antioxidant capacity, depending on heat magnitude, timing to expose heat, nature of antioxidant substances, and plant varieties[23].

Availability of antioxidant activity in Allium plants such as red and yellow onions, was reported to be closely correlated with the presence of total phenolic compounds, in which being highest at the outer layers and continuously decreased towards the inner layers[12-13]. Attempt to compare total phenolic compounds and antioxidant capacity of this study with other reports on Allium plants, was not reliable, since extracting procedure and assayed system were quite different. In rough assumption, antioxidant capacity and total phenolic compounds contained in shallot bulb samples of this study, were relatively high, comparing to the two Chinese varieties of yellow and red onions[12], and reports on green onions[24], and shallots[25].

In onions, amounts of phenolic derivatives such as quercetin-3,4′-O-diglucoside and quercetin-4′-O-monoglucoside, were reported to be higher significantly after storing for seven months at ambient conditions[22]. Up to the present times, analytical data of Srisaket variety's shallots have still to be limited. Thus, more investigations with longer storing times e.g. 6-12 months and different storing conditions, should be revealed. To the findings of this study, unchanged levels of total phenolic compounds and antioxidant capacity in matured shallot bulbs (Srisaket variety), remained for at least 3 months after harvesting, if stored at ambient conditions following villagers' preserving method.

Conclusions

A simply extracting method by using hot water (100°C) and extracting time of 60 minutes, were optimally used for extracting total phenolic compounds from shallot bulbs of Srisaket variety (*Allium acolonicum* L.). The whole bulbs contained 190.56- 221.34 mg/g dried weight of total phenolic compounds, and 0.113- 0.128 mg/g dried weight of antioxidant capacity. Levels of total phenolic compounds and antioxidant capacity of the peel part, were 4 and 2 times higher than those of the inner part, respectively. Allowing native shallot bulbs at ambient temperature conditions for three months, values of 3.76% of decayed bulbs and 16.61% of bulb weight loss, were observed. No significant change of antioxidant capacity and total phenolic compounds in the whole bulbs, inner and peel parts of shallot bulb samples, was shown. Analyzed data of this study lead to enlighten nutritional value of Srisaket variety's shallots, and be useful for further value-added applications.

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