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Research Article

### Bioactive compounds and antioxidant capacities of *phulae* and *nanglae* pineapple

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#### Abstract

Bioactive compounds and antioxidant capacities of “*Phulae*” and “*Nanglae*” pineapple (*Ananas comosus* L. Merr) which are important geographical indications of Chiang Rai, Thailand were investigated. Two pineapple varieties were collected at 20-40 % yellow peel colour. Total soluble solids (TSS), titratable acidity (TA), total polyphenols,  $\beta$ -carotene, vitamin C and antioxidant capacity (ferric reducing antioxidant power (FRAP) assay and 2, 2-diphenyl-1-picrylhydrazil (DPPH assay) were analyzed in the fruit pulp. The TSS, TA and TSS/TA contents of both varieties were not significantly different. “*Phulae*” pineapple had vitamin C content, total polyphenol and  $\beta$ -carotene higher than “*Nanglae*” pineapple. However the antioxidant capacities (DPPH-assay and FRAP-assay) of “*Phulae*” were found to be significantly lower than “*Nanglae*” pineapple. This result indicates that vitamin C, phenolic compounds and  $\beta$ -carotene do not play a major role in the antioxidant capacity of pineapples and thus it may be a consequence of other bioactive compounds.

**Keywords:** antioxidant capacity, bioactive compound, DPPH assay, TSS, TA, FRAP, Thailand

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#### Introduction

“*Phulae*” and “*Nanglae*” pineapple (*Ananas comosus* L. Merr) are important geographical indications of Chiang Rai Province, Thailand. “*Phulae*” pineapple refers to the Queen pineapple

variety. The fruit is small, weighing 150-1,000 grams; the skin is rather thick and suitable for long distance transport. When the fruit is ripe, the skin will be yellow or greenish yellow. The pineapple fresh colour is relatively light yellow, crispy and aromatic. The core is crispy and edible (Fig. 1-left). *Nanglae* pineapple refers to the Honey pineapple variety, a sub-variety of the Pattavia pineapple. The fruit is round and stout. The skin is thin. Its colour is green with some black or a mixture of yellow to dark orange, clearly bulging out at the eyes. The flesh is delicate with low fibre content. Its colour is yellow like honey. Fruit taste has a juicy sweetness than “*Phulae*” cultivar (Fig.1-right) [1]. Recently, both pineapple cultivars are becoming popular not only for local consumption, but also for export market. Considering the economic importance of Chiang Rai pineapple, it is surprising that study on their bioactive compounds and antioxidant capacities are limited.

Natural antioxidants, particularly in fruit and vegetables have gained increasing interest among consumers and researchers because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer [2]. The defensive effects of natural antioxidants in fruit and vegetables are related to three major groups; vitamin, phenolics and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants [3]. Pineapple fruit is considered a highly nutritious fruit because it contains a high level of vitamin C, a natural antioxidant which may inhibit the development of major clinical conditions including heart disease and certain cancers [4]. The fruit also contains phenolic compounds and  $\beta$ -carotene [5, 6], which constitute natural sources of antioxidants. Therefore information regarding antioxidants and antioxidant capacity of “*Phulae*” and “*Nanglae*” pineapples is needed for the benefit of consumers. The objective of this study is to investigate the physical and physicochemical characteristics, including bioactive compounds and antioxidant capacity of these two pineapple cultivars. The information obtained from this study will be useful for promoting and increasing fruit consumption and their economic value.



**Figure 1.** “*Phulae*” (left) and “*Nanglae*” (right) pineapple used for the study.

## **Materials and Methods**

### ***Plant Materials***

Two pineapple cultivars “*Phulae*” and “*Nanglae*” (*Ananas comosus* L. Merr) were harvested at the commercial maturity stage (20-40% yellow colour of fruit peel) from Chiang Rai, Thailand in October to November, 2008. Fruit of each cultivar consisted of four replications and there were

five fruits per replication. Fruit pulp was immediately frozen in liquid nitrogen and stored under -30°C until analysis of the total phenolic,  $\beta$ -carotene, vitamin C and antioxidant capacity.

#### ***Physical and physicochemical analysis***

Peel and pulp colour were measured on the middle part of the fruit by colorimeter (Color Quest XE Hunter Lab, USA) using the hunter scale ( $L^*$ ,  $a^*$  and  $b^*$ ) and expressed in hue and lightness ( $L^*$ ) values for fruit peel and pulp respectively. Pulp firmness was measured by texture analyzer TA-XT2 (Stable Micro System, Surrey, UK). Total soluble solids (TSS) were determined using a hand refractometer (ATAGO, Japan). Titratable acidity (TA) was measured on 1 ml juice titrated with 0.1 N NaOH and expressed as percentage of citric acid. Then pH of fruit juice was measured by pH meter (Eutech, pH 510). For moisture content analysis, fruit pulp was dried in a hot air oven at  $100 \pm 5^\circ\text{C}$  to a constant weight according to the Association of Official Analytical Chemists Method [7].

#### ***Vitamin C content***

Vitamin C content was analyzed by using liquid chromatography on an RP-Phase with UV detection according to Leong and Shui [8], with some modification. Standard solution of ascorbic acid at 5, 10, 20, 40, 80, 100 and 200  $\mu\text{g/ml}$  was prepared. Two grams of frozen pulp were homogenized in 18 ml of cold 3% meta-phosphoric acid and centrifuged at  $1500 \times g$  for 15 min at  $4^\circ\text{C}$ . Then the sample was immediately filtered through a Millipore membrane ( $0.2\mu\text{m}$ ) before injection. The separation was performed on a C18 column (250 x 4.6 mm i.d.) using 3mM  $\text{KH}_2\text{PO}_4$  in 0.35% *ortho*-phosphoric acid as the mobile phase at a flow rate 1.0 ml / min at  $30^\circ\text{C}$  oven temperature and the eluent was monitored at 248 nm. The vitamin C contents were expressed as milligram per 100 g fresh weight (FW) of pineapple.

#### ***$\beta$ -Carotene content***

$\beta$ -Carotene was analyzed according to the method of Queensland Health Scientific Service [9]. Ten grams of frozen pulp in 50 ml of petroleum ether were homogenized and centrifuged at  $2000 \times g$  for 20 min at  $4^\circ\text{C}$ . The supernatant were collected and the residue was twice extracted. The combined extracts were transferred to rotary evaporators and evaporated to dryness. The samples were then dissolved in 25 ml of *iso*-propanol and filtered through a nylon syringe filter ( $0.45\mu\text{m}$ ) prior to injection into HPLC. The analysis of  $\beta$ -carotene was performed using Agilent HPLC series HP 110 and a mobile phase mixture consisting of acetonitrile: methanol (70:30), with flow rate 1.6 ml/min. The separation was carried out on a Hypersil ODS C18,  $5\mu\text{m}$  (125 x 4.0 mm) at  $40^\circ\text{C}$  oven temperature and equipped with a Diode Array Detector monitoring at 450 nm.  $\beta$ -Carotene content in the fruit pulp was expressed as microgram  $\beta$ -carotene per 100 g FW of pineapple.

#### ***Total phenolic content***

Total phenolic content was measured using the Folin-Ciocalteu method described by Singleton *et al.* [10]. One millilitre of aqueous extract of pineapple pulp was added to 10 ml Folin-Ciocalteu reagent, followed by addition of 4 ml of an aqueous 7.5% solution of sodium carbonate. The mixture was stirred and allowed to stand for one hour. The solution was measured by spectrophotometer at 765 nm. A blank sample consisting of water and reagents was used as a reference. The results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g FW of pineapple.

**DPPH radical scavenging assay**

DPPH radical scavenging assay was determined based on the method described by Blois [11], with minor modifications. Fifty microlitres of aqueous extract of pineapple pulp were mixed with 2 ml of 60  $\mu$ M DPPH in methanol and allowed to stand in the dark for 60 min. Absorbance at 517 nm was measured using methanol as a blank. The control and standard were subjected to the same procedure as the sample except only distilled water was added to the control and the sample extracted was replaced with 0-1000  $\mu$ M Trolox as for a standard. The radical scavenging activity was calculated as described by Ribeiro [12]. Results were expressed as micromole Trolox equivalent (TE) antioxidant capacity per 100 g FW of pineapple.

**Ferric reducing antioxidant power (FRAP)**

The FRAP assay was undertaken according to Benzie and Strain [13], with some modifications. One millilitre of aqueous extract of pineapple pulp was mixed with 2.5 ml of phosphate buffer (0.2 M, pH6.6) and 2.5 ml of a 1% trichloroacetic acid was added and incubated at 50°C for 30 min. Then 2.5 ml of water, 0.5 ml of 0.1% aqueous FeCl<sub>3</sub> were added, mixed with vortex mixture and the absorbance was recorded at 700 nm. The control and standard was subject to the same procedure as the sample except only distilled water was added to the control. For the standard, the extract was replaced with 0-1000  $\mu$ M ascorbic acid standard. Iron (III) reducing activity was determined as micromole equivalent of ascorbic acid (AAE) per 100 g FW of pineapple.

**Statistical analysis**

Analysis of variance was performed by ANOVA procedures (SAS 8.0 for Windows). Significant differences were calculated according to the student's t test. Differences at  $P < 0.05$  were considered statistically significant.

**Results and Discussion**

The physical and physicochemical characteristics of “Phulae” and “Nanglae” pineapple are summarized in Table 1. The fruit pulp colour, firmness, pH and moisture content were significantly different between both pineapple cultivars, but there was no significant difference in fruit peel colour, TSS, TA and TSS/ TA ratios.

**Table 1. Physical and physicochemical characteristics of “Phulae” and “Nanglae” pineapple.**

Characteristics	Phulae	Nanglae
Peel colour (hue)	77.95 $\pm$ 2.47 a	75.10 $\pm$ 6.28a
Pulp colour (L)	61.03 $\pm$ 1.49b	70.99 $\pm$ 0.64a
Firmness (N)	17.19 $\pm$ 0.01a	15.64 $\pm$ 0.01b
TSS ( $^{\circ}$ Brix)	14.45 $\pm$ 0.18a	13.10 $\pm$ 0.43a
TA (%)	0.65 $\pm$ 0.03a	0.68 $\pm$ 0.08a
TSS/TA	22.60 $\pm$ 0.66 a	21.45 $\pm$ 2.92a
pH	3.55 $\pm$ 0.01b	4.56 $\pm$ 0.06a
Moisture content (%)	84.87 $\pm$ 0.18b	86.79 $\pm$ 0.52a

Values are the mean  $\pm$ SE of  $n = 20$  fruit.

Within a row, different letters show significant differences ( $P < 0.05$ ) between two cultivars.

Bioactive compounds and antioxidant capacities of “Phulae” and “Nanglae” pineapple are shown in Table 2. Vitamin C contents were 18.88 and 6.45 mg/100 g FW in “Phulae” and “Nanglae” respectively. There was almost three times higher vitamin C content in “Phulae” than “Nanglae”

cultivars. The  $\beta$ -carotene contents were found to be relatively low in both cultivars but they were significantly higher in “Phulae” than “Nanglae” cultivar. In addition, total phenolic contents were  $26.20 \pm 0.49$  and  $20.28 \pm 1.18$  mg GAE/ 100 FW in “Phulae” and “Nanglae” respectively. The amounts of these bioactive compounds have been widely reported in pineapple varieties with differences in their concentration [6, 14, 15]. Differences in the concentration may be due to a number of factors, including cultivars, natural variation of fruit, climatic conditions or soil, fertilizer or geographical origin [6]. Methods for sampling, preparing and determination also influences the amounts of these compounds in pineapple [15, 16].

There is not only vitamin C, carotenoids and phenolic compounds existing in pineapple and there might be other varieties of antioxidants contained in the fruit. Therefore, measuring the antioxidant capacity of each compound separately becomes very difficult. Several methods have been developed to estimate the antioxidant capacity of different plant material [17]. In this study two different methods have been used to evaluate the antioxidant capacity of the aqueous extract of two pineapple cultivars; they were DPPH radical scavenging assay and FRAP reducing power assay. It was found that “Nanglae” had significantly better scavenging ability than “Phulae” cultivar. The DPPH radical scavenging capacities in aqueous extract of pineapple pulp were  $152.93 \pm 10.51$  and  $118.18 \pm 8.19$   $\mu\text{mol TE}/100\text{g FW}$  for “Nanglae” and “Phulae” cultivars respectively. Moreover, both pineapple cultivars had reducing power. The FRAP value of “Nanglae” cultivar was  $205.73 \pm 9.15$   $\mu\text{mol AAE}/100\text{g FW}$  which showed it to be significantly higher in reducing power than “Phulae” ( $165.28 \pm 2.04$   $\mu\text{mol AAE}/100\text{g FW}$ ). In honey pineapple, the recovery of phenol, FRAP and DPPH values were dependent on the solvent extracted. The 50% acetone in aqueous solvent was the most efficient solvent for extracting phenols and giving highest FRAP value than the aqueous or other organic solvents [15]. It has also been suggested that antioxidant activities gave higher measurements in organic systems than in aqueous systems [18]. Therefore, the relationship between various extraction solvents and antioxidant capacity must be further investigated in both “Phulae” and “Nanglae” pineapple.

**Table 2. Bioactive compounds and antioxidant capacities of “Phulae” and “Nanglae” pineapple.**

Bioactive compounds and antioxidant capacities	Phulae	Nanglae
Vitamin C (mg/100 g FW)	$18.88 \pm 0.03\text{a}$	$6.45 \pm 0.68\text{b}$
$\beta$ -carotene ( $\mu\text{g}/100$ g FW)	$3.35 \pm 0.27\text{a}$	$1.41 \pm 0.01\text{b}$
Total phenolics (mg GAE/100g FW)	$26.20 \pm 0.49\text{a}$	$20.28 \pm 1.18\text{b}$
DPPH (( mol TE/100g FW)	$118.18$ (8.19b)	$152.93$ (10.51a)
FRAP (( mol AAE /100g FW)	$165.28$ (2.04b)	$205.73$ (9.15a)

Values are the mean  $\pm$  SE of n = 4 determinations.

Within a row, different letters show significant differences ( $P < 0.05$ ) between two cultivars.

The correlation coefficient between antioxidant capacities and bioactive compounds of “Phulae” and “Nanglae” pineapple were also investigated. It was obvious that the vitamin C,  $\beta$ -carotene and total phenolic contents showed a much higher correlation with FRAP reducing power ( $R^2 = 0.91$ ,  $0.71$  and  $0.78$ , respectively) than with the DPPH radical scavenging activity ( $R^2 = 0.60$ ,  $0.52$  and  $0.22$ , respectively). Moreover, vitamin C content strongly correlated with antioxidant capacity as determined by both DPPH radical scavenging activity and FRAP reducing power. No similar association was found for total phenolic content in DPPH radical scavenging assay.

In general, antioxidant activity is influenced by the amount of antioxidants in fruit. However, in this study it was found that the amount of these antioxidant compounds was not directly proportional to their antioxidant capacity, since “*Phulae*” contained higher amounts of vitamin C, carotenoids and total phenolics than “*Nanglae*”, but they were significantly lower in antioxidant activity. This result indicates that vitamin C, phenolic compounds and  $\beta$ -carotene do not play a major role for the antioxidant capacity of pineapples and it may be a consequence of other bioactive compounds. Similar findings have been observed by Hassimoto *et al.* [19] that the levels of phenolics, anthocyanins and flavonoids fluctuated in diverse plant extracts and there is no relationship between the content and its antioxidant activity. This suggests that antioxidant activity is the result of a combination of different compounds having synergistic and antagonistic effect.

### Conclusion

The results of this study indicate that both the “*Phulae*” and “*Nanglae*” pineapple varieties contain bioactive compounds and have antioxidant capacity as determined by DPPH and FRAP assay. The “*Phulae*” pineapple had a higher content of vitamin C, total phenolics and  $\beta$ -carotene than the “*Nanglae*” cultivar. However, the antioxidant capacity of “*Phulae*” was found to be significantly lower than the “*Nanglae*” cultivar.

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