# Research article

Comparison between the Physicochemical Properties, Bioactive Compounds and Antioxidant Activities of Thai and Chinese Garlics

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#### **Abstract**

# Keywords

allicin;

Allium sativum L.;

antioxidant activity;

consumer acceptance;

garlic

Garlic (Allium sativum L.) is an economically important vegetable that contains bioactive compounds and has high levels of antioxidant activity. Thus, it is commonly used in traditional medicine and culinary contexts. The aim of the present work was to compare the physiochemical and antioxidant properties and the bioactive compounds of three commonly used garlic cultivars in Thailand, the 'Srisaket', 'Chiangmai' and 'Chinese' cultivars. The results revealed that Srisaket garlic contained the highest amount of protein (32.96±0.51% dry weight), while Chiangmai garlic contained the highest amount of carbohydrate (73.81±0.44% dry weight). Chinese garlic contained the highest amounts of fiber  $(3.29\pm0.05\%$  dry weight) and ash  $(6.03\pm0.07\%$  dry weight) and the lowest amount of fat (0.15±0.01% dry weight). Antioxidative measurements indicated that Chinese garlic had the largest total phenolic content (543.8±13.4 mg gallic acid equivalent per 100g dry weight). However, Srisaket garlic contained the highest amounts of allicin (100.8±2.7 mg per 100 g dry weight) and total flavonoids (95.0±2.0 mg of quercetin equivalents), as well as the highest antioxidant activity (161.6±24.9 and 348.9±1.0 mg of trolox equivalents based on the DPPH and the ABTS assays, respectively). These results suggests that the Srisaket cultivar might confer greater health benefits than the Chiangmai and the Chinese cultivars. From the consumer acceptance test of fresh garlics (n=30), Thai consumers accepted all three cultivars with nonsignificant differences. The Srisaket cultivar was rated at 5.47±1.36 for overall liking because of its pungent flavor.

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#### 1. Introduction

Garlic (*Allium sativum* L.) is a major horticultural crop. It is grown throughout the world with a total harvested area of 1,437,690 hectares and an annual dry-bulb yield of 24,255,303 tons [1]. Garlic cultivation originated in central Asia and then spread to China, the Mediterranean region, Europe, Northern Africa and Mexico [2]. Garlic was well known to ancient people and has been used as a spice and food flavoring agent as well as a traditional medicine. Garlic was often used to treat intestinal disorders, flatulence, worms, respiratory infections, skin diseases, wounds, symptoms of aging, and many other conditions [3]. Garlic contains various groups of bioactive compounds such as organosulfur compounds, saponins, phenolic compounds, and polysaccharides, which are the major active components of garlic [4]. Sulfur-containing allicin (diallyl thiosulfinate) is the most well-known biologically active component in freshly crushed garlic cloves or extracts [5]. Allicin is formed as a result of the action of enzyme alliinase on the non-protein amino acid alliin [6]. The amount of thiosulfinates, which can affect garlic odor intensity and antibiotic properties, varies among the garlic cultivars, [7].

There is no conclusive evidence on when garlic first arrived in Thailand, but it is speculated that it was first grown in home gardens. Then the cultivation area began to expand from the household scale to the agricultural scale in the central region of Ratchaburi province and the suburbs of Bangkok. Subsequently, the garlic cultivation area was expanded to the North and some parts of the Northeast of Thailand. This was because the northern region was more suitable for garlic cultivation than the central region. More than 90% of the garlic plantations in Thailand are in the northern region [8]. Thailand growers classify garlic cultivars into three groups. These are the Early cultivar group (e.g., the Srisaket cultivar), the Medium cultivar group (e.g., the Chiangmai cultivar), and the Late cultivar group (e.g., the Chinese cultivar). According to a 2015 report, imported Chinese garlic had the most competitive price due to the free trade agreement between Thailand and China, and this resulted in a continuous decrease in garlic farming in Thailand [9].

In previous research, Austin *et al.* [10] studied the chemical composition and DNA fingerprints of various samples of Thai garlic oil from Srisaket, Lamphun, Phayao, Chiangmai and Maehongson provinces. They found that the garlic oil samples could be divided into two groups based on their chemical compositions, which were the Srisaket and North garlic groups. At the same time, Sommano *et al.* [11] studied the chemical compositions of garlic essential oils derived from the Thai, Chinese and Pingpong cultivars, and found that the chemical and genetic profiles of the Thai and the Chinese cultivars were similar. However, the antioxidant activities were not studied, and all the above studies were specific for garlic essential oils, and not for raw garlic.

Therefore, this study was designed to determine the variability in the physiochemical and antioxidant activities as well as the bioactive compounds of the Srisaket, Chiangmai and Chinese garlics. Additionally, sensorial acceptance and the purchasing intension of the three cultivars were compared.

### 2. Materials and Methods

#### 2.1 Materials and chemicals

Garlic (*Allium sativum* L.) was purchased from local markets in Chiangmai province (Muang Mai fresh market and Siam Makro Plc.) and Srisaket province (Jae Mam Phanit), Thailand, in September 2019. The garlic samples were kept at room temperature until use. Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Trizma® hydrochloride, L-cystein and 5,5'-dithiobis(2-nitrobenzoic)

(DTNB) were purchased from Sigma-Aldrich (USA). Anhydrous sodium carbonate, aluminum chloride, and potassium acetate were purchased from Loba Chemie (India). Ethanol was purchased from RCI Labscan Ltd. (Thailand). All other chemicals and reagents used in the experiments were analytical grade.

## 2.2 Preparation of garlic extracts

The fresh garlic was peeled and sized, and only clean whole cloves were selected to make up a 50 g quantity for each cultivar. Garlic was ground in a blender (HR2115, Philips, Indonesia) and mixed with 50% ethanol at a ratio of 1:10 (w/v). The mixture was extracted at 60°C for 90 min in a water bath shaker (Memmert, Germany), filtered through Whatman® 4 filter paper, and the filtrate was kept at -18°C for analysis.

## 2.3 Physiochemical property analysis

### 2.3.1 Color and pH measurements

The color values of the garlic samples were measured using a MiniScan EZ 4500L Spectrophotometer (Hunter Associates Laboratory, Inc., USA) calibrated with black and white porcelain reference plates. The uniform color spaces, CIE-LAB L\*, a\*, b\* were measured. L\* defines lightness from black (L\*=0) to white (L\*=100); a\* defines the greenness (-) to redness (+); and b\* defines blueness (-) to yellowness (+). The pH was measured using an electronic pH meter (Mettler Toledo FE20, Switzerland).

#### 2.3.2 Proximate analysis

The moisture, fat, crude fiber, ash, and carbohydrate contents of the garlic samples were determined according to the methods defined by the Association of Official Analytical Chemists [12]. The generic combustion method [13] was used to determine the crude protein content of the samples.

#### 2.4 Allicin content measurement

The allicin content measurement method was modified from Sariri *et al.* [14]. One ml of a garlic extract was mixed with 1 ml Trizma® hydrochloride (1M, pH 8) and incubated at room temperature for 15 min. Next, the sample was mixed with 5 ml of DTNB solution, which was prepared by mixing a solution of 2 mM DTNB and 50 mM sodium acetate and 1 ml of 20 mM L-cysteine. Next, the solution was adjusted to 100 ml with distilled water and analysed on a spectrophotometer (Genesys 10S UV-Vis, Thermo Fisher Scientific, USA) at 412 nm. The allicin content was calculated as mg allicin/100 g dry weight.

#### 2.5 Total phenolic content (TPC) and total flavonoid content (TFC) measurements

The TPC in each garlic extract was determined based on the method developed by Fukumoto and Mazza [15]. Briefly,  $500~\mu l$  of the extract was mixed with 2.5 ml of freshly prepared 0.2 M Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate solution. The solution was covered and left at room temperature for 1 h before its absorbance was measured at 760 nm. The TPC was determined against a standard curve of gallic acid and expressed as mg of gallic acid equivalent (GAE) per 100 g dry weight.

The TFC was determined based on the method described in Jang *et al.* [16]. Specifically, 430  $\mu$ l of the extract was mixed with 85  $\mu$ l of 10% w/v aluminum chloride solution in methanol, 85  $\mu$ l of 1 M potassium acetate, and 2400  $\mu$ l of distilled water. The mixture was then incubated at room temperature for 30 min followed by measurement of its absorbance at 415 nm. The TFC was determined against a standard curve of quercetin and expressed as mg of quercetin equivalent (QE) per 100 g dry weight. Both TPC and TFC assays were carried out in triplicate.

#### 2.6 Antioxidant activity analysis

This study employed two antioxidant activity assays: the DPPH and ABTS radical cation scavenging activity assays. Both assays are based on the electron transfer principle and have been used to measure the antioxidant capacity of foods. All measurements were carried out in triplicate.

#### 2.6.1 DPPH radical scavenging activity

The DPPH radical scavenging activity was determined using the method from Lu *et al.* [17] with minor modifications. Specifically, 300 µl of a garlic extract was mixed with 3000 µl of 0.208 mM DPPH solution. The mixture was incubated in the dark at room temperature for 30 min followed by the measurement of its absorbance at 515 nm. Trolox concentrations of 0-500 µmol/l were used as the calibrating standard. The antioxidant capacity of the sample was expressed as mg Trolox equivalent antioxidant capacity (TEAC) per 100 g dry weight.

#### 2.6.2 ABTS radical cation scavenging activity

The ABTS radical cation scavenging activity analysis was modified from the method developed by Re *et al.* [18]. Briefly, the ABTS•+ solution was prepared by mixing 7 mM ABTS solution in distilled water with 2.45 mM potassium persulfate, and the mixture was then kept in the dark at room temperature for 12 to 16 h. Next, the ABTS•+ solution was adjusted with 0.1 M of sodium phosphate buffer (pH 7.4) to an initial absorbance of 0.75±0.005 at 734 nm. The reaction was initiated by adding 2900 μl of the ABTS•+ solution to 100 μl of a garlic extract. Then, the mixture was incubated in the dark at room temperature for 10 min followed by the measurement of its absorbance at 734 nm. Trolox was used as the calibrating standard. The antioxidant capacity of the sample was expressed as mg TEAC per 100 g dry weight.

### 2.7 Sensory evaluation

Data were collected from thirty assessors (23 females and 7 males between the ages of 20-40 years old) familiar with sensory evaluation methodologies [19, 20] in the Faculty of Agro-Industry, Chiang Mai University, Thailand. Each panelist evaluated one gram of each garlic sample served in a cup labeled with a random 3-digit number. The sensory attributes including color, shape, size, garlic odor, pungent odor, garlic flavor, spicy flavor, hardness, aftertaste and overall acceptance were rated on a 9-point hedonic scale (ranging from 1 = dislike extremely to 9 = like extremely). After tasting each sample, panelists cleaned their mouths by eating a piece of apple followed by a piece of white bread. Then they waited at least 2 min before evaluating the next sample. The panelists were also asked about acceptance and purchase intention. The consumer sensory panel was done in a self-contained panel booth at Sensory Evaluation and Consumer Testing Unit, Faculty of Agro-Industry, Chiang Mai University, which complied with international standards (ISO).

#### 2.8 Statistical analysis

All experiments were conducted in triplicate. Basic statistical parameters such as means and standard errors of the mean were determined using SPSS Statistics 17.0 for Windows (SPSS Inc. Chicago, Illinois, USA). Differences among the garlic cultivars were determined using one-way ANOVA with Duncan's multiple range post-hoc analysis at the 95% confidence level.

#### 3. Results and Discussion

# 3.1 The physiochemical properties of the three garlic cultivars

All three cultivars of garlic had yellowish white color with Chinese garlic showing the most intense color (Table 1 and Figure 1). Srisaket and Chinese garlic was brighter (indicated by L\* values) than Chiangmai garlic. Lightness has been shown to be a useful discriminating parameter among garlic cultivars. Chinese garlic was redder (indicated by a\* values) and yellower (indicated by b\* values) than the others (p≤0.05). This difference in the intensity of yellowness and redness may be due to different amounts of carotenoids in the samples. Carotenoids contribute to the yellow and red colors in many fruits and vegetables [21], and it has been suggested that carotenoids are largely responsible for the color of garlic. These observations were likely to be explained by growth environment rather than cultivar type [22]. Chinese garlic had the lowest pH at 6.14±0.02, followed by Srisaket (6.39±0.01) and Chiangmai (6.45±0.01), respectively. This indicates that Chinese garlic was more acidic than Srisaket and Chiangmai garlic. Pardo et al. [23] reported that colors were linked with pH values as darker garlic tended to have a higher pH and negatively correlated with L\* and b\* values, which was found for the purple cultivar. On the contrary, the white cultivar had the lowest pH, which was positively correlated with L\* and b\*. However, the Chiangmai cultivar had higher pH but lower L\* and b\* than the Srisaket and Chinese cultivars in this study. Furthermore, this cultivar was visually the whitest in color (Figure 1).

The nutritional composition of each garlic cultivar was determined using proximate analysis (Table 2). The average moisture content of the three garlic cultivars were 65-70%. Srisaket garlic had the highest mean protein content of all cultivars at 32.96±0.51% dry weight, which is consistent with the results of previous studies [24]. The existence of the protein may be due to the presence of alliin, which is found naturally in garlic and converted to biologically active proteins such as allicin and ajoene [25]. Srisaket garlic had a similar fat content to that of Chiangmai's (mean±SD; 0.82±0.13 and 0.90±0.08% dry weight, respectively), although the latter contained a higher amount of carbohydrate (73.81±0.44% dry weight). Although garlic generally has a low fat content and does not qualify as an oil plant, the oil can be extracted for use as an essence or an essential oil [26] that contains several sulfur-containing compounds, particularly allyl polysulfides, such as diallyl disulfide, allyl methyl trisulfide and diallyl trisulfide [19, 27]. Garlic oil from garlic grown in different geographic regions and climates was shown to have quantitative differences but not qualitative differences in terms of organosulfur compounds [21]. The higher carbohydrate content of Chiangmai cultivar could make it a better source of energy for the body [28] than Srisaket and Chinese cultivars.

It is noteworthy that Chinese garlic contained the largest amounts of crude fiber and ash (3.29±0.05 and 6.03±0.07% dry weight, respectively). The level of ash in garlic, which includes inorganic minerals, is relatively low compared to other vegetables [29]. However, the minerals in garlic, such as calcium, potassium, phosphorus, iron, zinc and magnesium, are nutritionally

**Table 1.** The pH and color of raw garlic from three cultivars. The mean±SD for each cultivar was generated from three independent samples.

Garlic cultivars	рН	Color		
		L*	a*	b*
Srisaket	6.39±0.01 <sup>b</sup>	68.24±0.58a	-1.17±0.04 <sup>b</sup>	36.78±0.21 <sup>b</sup>
Chiangmai	$6.45{\pm}0.01^a$	$64.05\pm0.04^{c}$	$-1.28\pm0.05^{c}$	35.79±0.11°
Chinese	$6.14\pm0.02^{c}$	$67.33\pm0.07^{b}$	$-0.28\pm0.04^{a}$	38.39±0.11a

**Note:** Different superscripts in the same column represent significant differences ( $p \le 0.05$ ) among cultivars.



Figure 1. Representative samples of Srisaket, Chiangmai and Chinese garlic cultivars

**Table 2**. The proximate composition of raw garlic samples from three cultivars. Means±SDs were shown as % dry weight.

Composition		Garlic cultivars	
(% dry weight)	Srisaket	Chiangmai	Chinese
Moisture	70.00±0.18 <sup>b</sup>	65.05±0.04°	70.41±0.05a
Crude fat	$0.82 \pm 0.13^{a}$	$0.90{\pm}0.08^a$	$0.15\pm0.01^{b}$
Protein	$32.96\pm0.51^{a}$	$18.70 \pm 0.48^{c}$	$20.08\pm0.64^{b}$
Carbohydrate	$57.94\pm0.48^{c}$	$73.81 \pm 0.44^{a}$	$70.45 \pm 0.66^{b}$
Crude fiber	$3.13\pm0.07^{b}$	$2.54\pm0.09^{c}$	$3.29\pm0.05^{a}$
Ash	$5.15\pm0.04^{b}$	$4.06\pm0.11^{c}$	$6.03\pm0.07^{a}$

**Note:** Different superscripts in the same row represent significant differences (p≤0.05) among cultivars.

important [25] as they have a significant role in the control of arterial resistance and regulation of fluid balance in the body, and thus may influence cardiac output [30].

#### 3.2 Groups of bioactive compounds present in the three garlic cultivars

Great variability in TPC, TFC and allicin content was observed among the garlic cultivars (Figure 2). The Chinese cultivar had the highest level of TPC (Figure 2a), followed by the Srisaket and the Chiangmai cultivars, respectively (543.8±13.4, 380.9±3.4 and 266.6±6.6 mg GAE/100 g dry weight). Beato *et al.* [31] found that caffeic acid and ferulic acid were the main phenolic acids in garlic. However, Chinese garlic had the lowest level of TFC among the three cultivars, while Srisaket garlic contained the highest level (46.5±4.2 vs. 95.0±2.0 mg QE/100 g dry weight) (Figure 2b). The different flavonoid content may be related to the cultivars' harvesting ages. Flavonoids are

natural secondary metabolites that are synthesized using photosynthetic products. Excessive growth of the plants can reduce the synthesis of flavonoids because larger trees consume more photosynthetic products, and thus fewer resources are available for flavonoid biosynthesis [32]. The Srisaket cultivar had shorter harvesting age (75-90 days) than the Chiangmai (100-120 days) and Chinese cultivars (150 days) [33]. Therefore, the Srisaket garlic plants may require fewer resources for photosynthesis and were able to allocate their carbon supply and energy to the production of flavonoids. Other potential explanations for the differences in the amounts of TPC and TFC include genetic diversity and environmental variation [34].

In addition to flavonoid content, the Srisaket cultivar contained the highest amount of allicin (100.8±2.7 mg allicin/100 g dry weight) (Figure 2c). This could be related to the high protein content detected in this study, because allicin is an active protein-like metabolite. Allicin easily decomposes into sulfur-containing volatile compounds, which are the main source of the flavor of garlic. Allicin is also a physiologically active molecule with many potential health benefits such as antimicrobial activity, anticancer and antioxidant activities, and immune function improvement [35]. Allicin was also shown to reduce serum protein levels and weights in albino rats, and reduce cholesterol and triglyceride levels, and plaque formation in blood [36]. Thus, allicin may potentially confer benefits with regards to cardiovascular disease. However, allicin content was affected by the cultivar type, climate conditions, and dormancy period [37].

#### 3.3 Antioxidant activity of the three garlic cultivars

The antioxidant activity was measured using DPPH and ABTS assays (Figure 2d). The results indicated that Srisaket garlic had the highest level of DPPH radical scavenging activities (161.6±24.9 mg TEAC/100 g dry weight) compared to Chiangmai (51.7±4.8 mg TEAC/100 g dry weight) and Chinese garlic (96.4±4.5 mg TEAC/100 g dry weight). The Srisaket cultivar also had a higher level of ABTS activity (348.8±1.0 mg TEAC/100 g) than the Chiangmai cultivar (302.1±0.8 mg TEAC/100 g dry weight). These findings agree with the allicin and TFC results because allicin exhibits radical-trapping activity after reacting with free thiol containing enzymes, thereby inhibiting formation of hydroxyl and superoxide radicals and subsequently nitric oxide formation [35]. Like flavonoids, they are oxidized by free radicals. As a result, free radicals become more stable and less reactive, helping to protect against cell injury [38].

## 3.4 Sensory acceptance

The results of the sensory evaluation showed differences in shape, size, garlic odor and pungency odor but no differences in color, garlic flavor, spicy flavor, hardness, aftertaste and overall acceptance (p>0.05) among the three cultivars (Table 3). For all of the differing characteristics, the Chinese cultivar had the highest preference scores and performed significantly better than the Chiangmai and Srisaket cultivars in all attributes except pungent odor preference for which the Chinese and Chiangmai cultivars were similarly preferred.

The results of these sensory evaluations were consistent with the panelist acceptance and purchase intention, as shown in Table 4. The majority of panelists accepted all three cultivars, with the Chinese cultivar being the most accepted (96.7%), followed by the Chiangmai (93.3%) and the Srisaket cultivars (76.7%). As for purchase intention, the largest proportion of the panelists stated that they would buy Chinese garlic (70.0%), followed by Chiangmai (56.7%) and Srisaket (50.0%) garlic. These results indicate that the Srisaket cultivar was least favored by the panelists, especially for its garlic and pungent odors, resulted in a low purchase intention, although it was high in allicin (allicin gives a strong pungent odor) [39], other bioactive compounds and antioxidant activities.

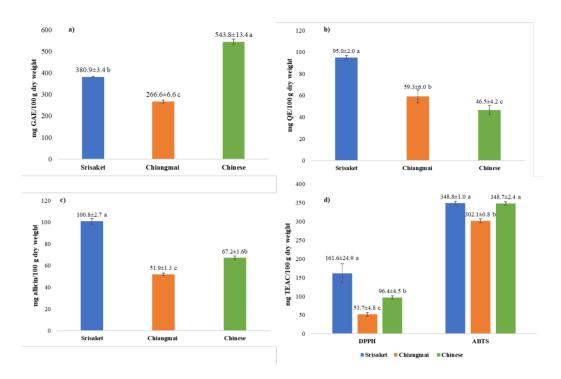


Figure 2. Total phenolic acid content (a), total flavonoid content (b), allicin content (c), and antioxidant activities of the Srisaket, Chiangmai and Chinese garlic (d)

Note: Bars and whiskers represent means±SDs respectively (n=3) with different lowercase letters indicating significant differences (p≤0.05) among cultivars. Abbreviations: Gallic acid equivalents, GAE; Quercetin equivalents, QE; Trolox equivalent antioxidant capacity, TEAC.

**Table 3.** Sensory preference scores: color, shape, size, garlic odor, pungent odor, garlic flavor, spicy flavor, hardness, aftertaste and overall acceptance. Different superscripts on the same row represent significant differences ( $p \le 0.05$ ) among cultivars. (n = 30)

Attributes	Srisaket cultivar	Chiangmai cultivar	Chinese cultivar
Color	$6.67{\pm}1.30^{ns}$	$6.53\pm1.33^{ns}$	$7.10\pm1.30^{ns}$
Shape	$6.53\pm1.31^{b}$	$6.30 \pm 1.49^{b}$	$7.27{\pm}1.36^{a}$
Size	$6.33 \pm 1.18^{b}$	$6.37 \pm 1.52^{b}$	$7.10\pm1.35^{a}$
Garlic odor	5.50±1.33°	$6.13\pm1.53^{bc}$	$6.40{\pm}1.50^{a}$
Pungent odor	$4.97 \pm 1.63^{b}$	$5.93{\pm}1.55^a$	$6.00{\pm}1.66^a$
Garlic flavor	$5.10\pm1.69^{ns}$	$5.47{\pm}1.53^{ns}$	$5.53{\pm}1.82^{ns}$
Spicy flavor	$4.90{\pm}1.71^{ns}$	$5.77 \pm 1.63^{ns}$	$5.63\pm2.01^{ns}$
Hardness	$5.87{\pm}1.33^{ns}$	$6.37{\pm}1.25^{ns}$	$6.17 \pm 1.21^{ns}$
Aftertaste	$5.37{\pm}1.65^{ns}$	$5.47{\pm}1.53^{ns}$	$5.40{\pm}1.75^{ns}$
Overall acceptance	$5.47{\pm}1.36^{ns}$	$5.73\pm1.62^{ns}$	$6.03 \pm 1.75^{ns}$

**Note:** Different superscripts in the same row represent significant differences (p<0.05) among cultivars.

**Table 4.** Percentage (%) of panelist acceptance and purchase intention (n=30)

Garlic cultivars —	% Acceptance		% Purchase intention	
	Accept	Reject	Purchase	Not Purchase
Srisaket	76.7	23.3	50.0	50.0
Chiangmai	93.3	6.7	56.7	43.3
Chinese	96.7	3.3	70.0	30.0

#### 4. Conclusions

In this survey, we examined the physiochemical properties, bioactive compounds and antioxidant activities of Srisaket, Chiangmai and Chinese garlics because these cultivars are popular amongst consumers in Thailand. The results shows that Chinese garlic contained higher levels of fiber, ash, and total phenolic compounds compared to Srisaket and Chiangmai garlic. Srisaket garlic had the highest levels of protein, allicin, and total flavonoids as well as the greatest antioxidant activity among the cultivars. Chiangmai garlic had relatively inferior physiochemical and antioxidant properties. Overall, the study suggests that Srisaket garlic possesses the greatest health-promotion potential. The higher levels of allicin and antioxidant activity present in Srisaket garlic represent an opportunity to market fresh garlic to panelists based on its health properties. However, genetic diversity may not be the only factor affecting the garlic compositions and attributes. Other potential factors included here are geographic regions, environmental variation, harvesting ages, etc. Moreover, we found that Srisaket cultivar was less accepted by potential consumers, who also showed a lower intention to buy it than Chiangmai and Chinese cultivars. Therefore, we recommend that there should be further development of product from Srisaket cultivar in order to get more acceptable to consumers.

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