

RESEARCH ARTICLE

Nutritional Composition, Capsaicin Content and Enzyme Inhibitory Activities from “Bang Chang” Thai Cultivar Chili Pepper (*Capsicum annuum* Var. *acuminatum*) after Drying Process

Kanyapat Petcharaporn¹, Kanittada Thongkao¹, Pimporn Thongmuang², Yuttana Sudjaroen^{1*}

¹Department of Applied Science, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok, Thailand

²Department of Aesthetic Health Science, College of Allied Health Sciences, Suan Sunandha Rajabhat University, Samut Songkhram, Thailand

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ABSTRACT

Chili pepper cultivars come in a broad variety of tastes, colors, sizes, and spiciness levels due to various growth conditions. The significance of different chili peppers and other spices in Thai cuisine may be seen in dishes. This study evaluated the nutritional composition and capsaicin content from “Bang Chang” Thai cultivar chili pepper after drying, and the inhibition of lipase, tyrosinase and elastase enzymes from its oil and ethanol extracts. The nutritional composition of sun-dried chili pepper was high dietary fiber and beta-carotene, which were significantly high amount portion of Thai recommended dietary intake (RDI). According to calculations and definitions, “Bang Chang chili pepper” is a non-pungent *Capsicum* (0-700 SHU) since it contains a low amount of capsaicin. Total phenolic content was more abundant in ethanol extract (2.50 ± 0.13 mg GAE/g) than oil extract (1.05 ± 0.05 mg GAE/g). Compared to the positive control orlistat (IC₅₀ = 3.26 ± 0.28 mg/mL), an anti-lipase drug, ethanol extract was 2.0 times more lipase inhibitory. In addition, ethanol extract was mildly anti-tyrosinase, while oil extract was non-activity. The anti-lipase activity of ethanol extract was due to phenolic content rather than capsaicin contained and this enzyme inhibition may act on active site. We found that ethanol extract of “Bang Chang” cultivar chili pepper yielded higher TPC content and more effective anti-lipase than oil extract. The finding provided benefits on applying this chili pepper on anti-lipase use and weight management.

Keywords: *Capsicum annuum* var. *acuminatum*, Chili pepper, Anti-lipase, Anti-tyrosinase.

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INTRODUCTION

Chili peppers (*Capsicum* spp.) are a very significant crop in many Asian nations, including Bangladesh, China, India, Indonesia, Thailand, and Vietnam. In Asian food traditions, agricultural and economic factors are key since they serve as an essential spice.^{1,2} In Thailand, the climatic and geographical circumstances of the agricultural areas differ. The northern, central, and southern regions have mountain ranges, river plains, and coastal areas. Chili pepper cultivars come in a broad variety of tastes, colors, sizes, and spiciness levels due to various growth conditions.^{3,4} Nutritional composition among chili peppers (per 100 g) were also varied, including calories (40–50 kcal), carbohydrates (8–9 g), dietary fiber (2–3 g), protein (1–2 g), fat (less than 1 g) and potassium (250–400 mg). Vitamin C and vitamin A (as beta-carotene) from chili peppers are highly variable. Therefore, there a significantly

high amount portion of recommended dietary intake.^{5,6} The unique bioactive components found in chili peppers, capsaicin and capsaicinoids are responsible to pungent level or spiciness. scoville heat units (SHU) are used to measure and describe the spiciness of chili peppers.⁶⁻⁸ The significance of different chili peppers and other spices in Thai cuisine may be seen in dishes like “Tom Yum Goong,” a fiery shrimp soup, and “Som Tum,” a green papaya salad. Significant quantities of chili peppers are exported to several Asian nations with robust culinary industries. As many processed preserved goods, such as sauces, pastes, and dried forms, are also available.^{3,6-9}

Capsaicin in chili peppers is plays a role of chemoprevention and physiological modulation i.e., pain relief, increase fat metabolism on weight management, stimulate digestion, improve blood circulation, reduce cholesterol and antioxidant properties. The physiological modulation of capsaicin has

*Author for Correspondence: yuttana.su@ssru.ac.th

been studied for inhibitory effects on various enzymes such as the transient receptor potential vanilloid 1 (TRPV1) receptor, enzymes involved in pain perception, metabolic enzymes, and inflammatory enzymes.^{7,8,10-15} In addition, capsaicin may exhibit the activity of lipase enzymes. This inhibition indirectly reduces fat absorption and digestion in the gastrointestinal tract. By interfering with fat digestion, capsaicin might contribute to weight management efforts and have implications for metabolic health. This might contribute to apply on weight management and control metabolic impairment, such as glucose metabolism.¹⁶⁻¹⁸ Inhibition of tyrosinase and elastase enzymes of capsaicin may apply on skincare purposes, which are regulate skin hyperpigmentation and elasticity.¹⁹⁻²³ The Thai cultivar of capsicum, known as the “Bang Chang chili pepper” (*C. annuum* var. *acuminatum*) was initially grown in Thailand’s Bang Chang subdistrict, Samut Songkhram. The antioxidant activity and nutrient content had already been shown.^{24, 25} Food preservation is the process of preventing or slowing down the spoilage and deterioration of food. In Asian culture, chili peppers are favored to preserve by drying which removes moisture and inhibits the growth of spoilage microorganisms. This study aimed to evaluate nutritional composition and capsaicin content from “Bang Chang” Thai cultivar chili pepper after the drying process, and inhibition of lipase, tyrosinase and elastase enzymes from its oil and ethanol extracts. The finding may provide necessary information on utilizing this Thai cultivar chili pepper in healthcare.

MATERIALS AND METHODS

Pepper Production, Harvesting and Extraction

The “Bang Chang” cultivar chili pepper (*C. annuum* var. *acuminatum*) seeds were provided by the Tropical Vegetable Research Center at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand, and were then planted in the Samut Songkhram Campus of Suan Sunandha Rajabhat University, Thailand, as the original area, from November 2022 to February 2023. Plant identification has been validated by earlier study.^{24, 25} Red peppers or ripe fruits were harvested to make sun-dried chili peppers. Chili peppers that had under 1% moisture content were considered sufficient because they had been sun-dried. Fruit pedicels were cut off, the remaining material was pulverized, and the fruits were then separated. Oil extract was explained that one liter of rice bran oil (RBO) was macerated with 500 g of chili pepper powder over the course of three days. Whereas ethanol extract was macerated chili pepper powder as similar ratio.

Each extract was filtered to remove impurities, evaporated to constant weight, and kept into an amber glass bottle at 4°C.

Nutritional Composition

Within three days, finely ground sun-dried chili peppers were delivered to a lab. The samples were combined, and triplicates were examined. The Central Laboratory Co., Ltd., Bangkok, Thailand, performed the nutritional value analysis. According to AOAC International,²⁶ nutritional analysis was performed

on values for ash, calories, carbs, protein, total dietary fiber, total sugars, fatty acids, salt, calcium, iron, vitamin A, and vitamins B1 and B2. Sun-dried chili pepper nutrition content per 100 g of serving size was reported and analyzed using Thai RDIs.²⁷ Descriptive statistics were used to examine and display all of the data.

Capsaicin Content

According to the official AOAC procedure, dried chili pepper was made with acetone and ethanol. A ZORBAX C18 (4.6 250, 5 mm)-equipped Agilent HPLC system was used to measure the capsaicin content. For 15 minutes, a sample (10 µl) was injected into a mixture of methanol and water (70:30 v/v) at a flow rate of 1.0 mL/min. The signal was represented by a photodiode array detector and mass spectrophotometer as a chromatogram and mass spectrum, respectively, which corresponded to standard capsaicin. Capsaicin was retained for around 8.5 minutes at UV wavelengths ($\lambda_{\text{max}} = 228$ and 280 nm). By comparing the amount of capsaicin in each sample to a known amount (Sigma, USA), the amount of capsaicin was determined.^{28,29} Capsaicin content in ppm ($\times 15$) was used to approximation the pungency level in SHU.³⁰

Total Phenolic Content

The Folin-Ciocalteu test was used to calculate the total phenolic content (TPC). Dimethylsulfoxide (DMSO) was used to dissolve the extract before adding the Folin-Ciocalteu reagent and the appropriate alkaline solution. A spectrophotometer set at 760 nm was used to measure the amount of blue color that resulted, which was proportionate to the total phenolic content. The amount was calculated as mg of gallic acid equivalents per gram of extract (mg GAE/g).³¹

In-vitro Enzyme Inhibition Assays

Test for anti-lipase

In 10% (v/v) DMSO was used to modify the concentration of each pepper extract to 0.001, 0.01, 0.1, 1 and 10 mg/mL. Additionally, 10% (v/v) DMSO was used to modify the dosage of orlistat (positive control), which was 0.0005, 0.005, 0.5, and 5 mg/mL. Monitoring the absorbance of p-nitrophenol, a result of p-nitrophenyl butyrate catalyzed by pancreatic lipase, at its maximum value at $\lambda_{\text{max}} = 415$ nm during assessment of the enzyme’s inhibitory action. The IC₅₀ of extract against pancreatic lipase was determined from triplicate measurements of the enzyme inhibitory activity from each pepper extract.³²

Test for anti-tyrosinase

In 10% (v/v) DMSO was used to alter the concentration of each pepper extract to 0.001, 0.01, 0.1, 1 and 10 mg/mL. The dopachrome technique was used to assess the anti-tyrosinase activity of extract. Micro-titer plate reader used to track enzyme inhibition of dopachrome reduction absorbance. The results, which were computed using a reference method and three separate measurements of absorbance, were reported as the IC₅₀ of extract against the tyrosinase enzyme. The positive control was kojic acid.³³

Test for anti-elastase

In 10% (v/v) DMSO was used to alter the concentration of each pepper extract to 0.001, 0.01, 0.1, 1 and 10 mg/mL. The positive control, epigallocatechin gallate (EGCG), was adjusted with Tris-HCl buffer at the same concentration range. By using a micro-titer plate reader, elastase enzyme inhibition was observed as an absorbance reduction of p-nitroanilide. P-nitroanilide is a byproduct of the elastase enzyme hydrolyzing N-succinyl-Ala-Ala-Ala-p-nitroanilide. The results, which were computed using a reference method and three separate measurements of absorbance, were reported as the IC₅₀ of the extract against the elastase enzyme.³⁴

RESULTS AND DISCUSSION

The nutritional composition of sun-dried chili pepper was depicted as 100 g per serving size (Table 1). Previous studies showed a high dietary fiber and beta-carotene, which were significantly high amounts of Thai recommended dietary intake (RDI).^{5,6,35} According to calculations and definitions, “Bang Chang chili pepper” is a non-pungent capsicum (0–700 SHU) since it contains a low amount of capsaicin.^{29,30} TPC was 1.5 times more abundant in ethanol pepper extract (EP) than oil pepper extract (OP). Compared to the positive control orlistat, an anti-lipase drug, EP was 2.0 times more lipase inhibitory. In addition, EP was mildly anti-tyrosinase, while OP was non-activity (Table 2).

As a risk factor for illnesses like cardiovascular, hypertension, type II diabetes, and various types of cancer, obesity is a concern for worldwide public health. The warning issued by the World Health Organization^{36, 37} shows the seriousness of obesity-related diseases. Dietary fats are broken down by lipases into smaller molecules and absorbed to the body. Anti-lipase refers to drugs or compounds that prevent the activity of lipase enzymes. The absorption of dietary fats can be slowed by reducing lipase activity, which may eventually reduce caloric intake and weight loss. Foods containing various bioactive components, including polyphenolic compounds, have unique positive benefits. Several processes, such as the interaction and inhibition of digestive enzymes like pancreatic lipase (EC 3.1.1.3), may account for the anti-obesity effects of polyphenolic substances.^{38, 39} Spices are an illustration of a bioactive-rich food that has been noted for its favorable impacts on human health. Hot pepper polyphenolic components are known for their antioxidant and anti-obesity activities, which are linked to an increase in energy expenditure.^{40, 41} There are a number of flavonoids like quercetin and luteolin as well as phenolic acids like p-coumaric and caffeic acids. Additionally, hot peppers contain capsaicin, a substance-specific to the capsicum genus that gives them their pungent flavor.^{42, 43} Four phenolic compounds found in hot peppers—caffeic acid, p-coumaric acid, quercetin, and capsaicin—were examined and compared to hot pepper extract for their ability to inhibit pig pancreatic lipase activity as well as their binding properties. According to molecular docking experiments, capsaicin is bonding far from the active site, whereas caffeic acid, quercetin,

Table 1: Nutritional composition and capsaicin content of Bang Chang” cultivar chili pepper

Nutrition value	Unit	Per 100 g	Per serving size	RDI (%) *
Energy	kcal	62.48	60	-
Energy from fat	kcal	3.24	0	0
Total fat	g	0.36	0	0
Saturated fat	g	0.08	0	0
Cholesterol	mg	ND	0	0
Protein (%N × 6.25)	g	2.19	2	-
Total carbohydrate	g	12.62	13	4
Dietary fiber	g	5.08	5	20
Sugar	g	3.05	3	-
Sodium	mg	12.64	15	1
Vitamin A	µg	261.14	(261.14)	35
Beta-carotene	µg	1566.85	(1566.85)	-
Vitamin B1	mg	0.053	(0.05)	4
Vitamin B2	mg	0.145	(0.14)	8
Calcium	mg	15.70	(15.70)	<2
Iron	mg	0.59	(0.59)	4
Capsaicin	mg/100 mL	0.018	-	-

*Recommended dietary allowance (RDI) is according to Ministry of Public Health, Thailand³⁵
ND = not determined

Table 2: Total phenolic content and enzyme inhibitory activities of dried pepper extracts

Sample/Assay	TPC ^a	LP ^b	TYN ^b	ELT ^b
(units)	GAE/g	mg/mL	mg/mL	mg/mL
EP	2.50 ± 0.13	1.60 ± 0.19	>1000	ND
OP	1.05 ± 0.05	ND	ND	ND
Orlistat		3.26 ± 0.28	-	-
Kojic acid		-	0.01 ± 0.00	-
EGCG		-		0.20 ± 0.01

^aTotal phenolic content (TPC) was mg of gallic acid equivalent (GAE) per g; ^benzyme inhibitory activities were demonstrated as 50% of inhibitory concentration, IC₅₀ (mg/mL). EP = pepper extracted in ethanol; OP = pepper extracted in rice bran oil; EGCG = epigallocatechin gallate; LP = anti-lipase activity; TYN = anti-tyrosinase activity; ELT = anti-elastase activity; ND = Not determine

and p-coumaric acid are inhibiting this enzyme at active site.⁴⁴ The most often used method for extracting capsaicin from capsicum is traditional solvent extraction, and the methods depend on a number of factors, including the kind and amount of solvent, the ratio of solvents, the length, the polarity, and the phases of extraction. Hence, we found that ethanol extract of “Bang Chang” cultivar chili pepper yielded higher TPC content and more effective anti-lipase than oil extract. The finding provided benefits on applying this chili pepper on anti-lipase use and weight management.

CONCLUSION

Capsaicin, phenolic compounds and enzyme inhibitory activity of ethanol and oil extracts from the “Bang Chang” Thai cultivar chili pepper (*C. annuum* var. *acuminatum*) after drying process were defined as non-unpungent. In the ethanol extract, phenolic compounds and anti-lipase properties were preferred. This pepper extraction can be taken as a nutritional supplement to aid with weight loss.

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