International Food Research Journal 21(6): 2237-2243 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

# Determination of major and minor capsaicinoids in different varieties of the *Capsicum* fruits using GC-MS and their inhibition effect of the chilli extract on α-amylase activity

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**Article history**

*Received: 27 November 2013*
*Received in revised form: 7 April 2014*
*Accepted: 9 April 2014*

**Keywords**

*Capsaicinoids*
*Chilli*
*α-amylase activity*
*Dinitrosalicylic acid*
*GC-MS*

**Abstract**

The major and minor components of capsaicinoids were determined in seven main varieties of the *Capsicum* fruits grown in Northeast of Thailand. The methanol extract of capsaicinoids was cleaned up by C18 cartridge prior to qualitative and quantitative analysis by GC-MS. The chilli samples qualitatively comprised of nine identified compounds including nornordihydrocapsaicin, nordihydrocapsaicin, N-vanillyl-nonanamide, capsaicin, dihydrocapsaicin, N-vanillyl-decanamide, homocapsaicin, homodihydrocapsaicin isomer I and isomer II. Using full scan MS, the analytical figures of merit of the method had been validated and, based on the calibration curve of dihydrocapsaicin, both groups of the capsaicinoids could be quantitatively determined. It was found that the two major capsaicinoids consisting of 88.3 – 96.6% (capsaicin, 45.2 – 60.5% and dihydrocapsaicin, 32.2 – 44.0%) were found in higher contents in all studied samples, while some of the minor ones were still undetectable. The total contents of these capsaicinoids were ranged of 3,718.2 – 7,671.8 μg $g^{-1}$ DW. Furthermore, an inhibition of α-amylase activity of the chilli extract was estimated by dinitrosalicylic acid method, resulted as a potent inhibitor (16 – 33% inhibition) against the amylase activity, potentially regarding to physiological response for a risk reduction of diabetes.



## Introduction

Chilli or chilli peppers (*Capsicum* spp.) are popular spices in many parts of the world. Their properties of color, aroma, flavor and pungency account for their extensive usage. The pungency is the most outstanding property of the so-called "hot" chilli pepper, resulting from the accumulation of capsaicin and other related compounds commonly known as capsaicinoids. They are organic compounds which are only found in the *Capsicum* genus and bioactive molecules currently relevant in medical and food science (Caterina *et al*., 2000; Chu *et al.*, 2003). Its chemical structure is composed of a vanillylamide moiety and an acyl chain of 8-13 carbon atoms (Schweiggert *et al*., 2006). Two major capsaicinoids present in most varieties of hot chilli are capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-N-vanillylnona-namide) as shown Figure 1. In addition to the two major components, other minor capsaicinoids are also existed in chilli, including nordihydrocapsaicin, norcapsaicin, homocapsaicin, homodihydrocapsaicin, nornorcapsaicin, nornornorcapsaicin, and nonivamide (Barbero *et al*., 2008). Some minor capsaicinoids have also been identified but are present at very low levels and are not thought to make any contribution greatly to the overall pungency of the capsicum fruits (Lopez-Hernandez *et al*., 1996). The capsaicinoids have broad bands of both properties and their corresponding applications. In particular, they are antimutagenic and antitumoral compounds (Surh and Lee, 1996), antioxidants (Barbero *et al.*, 2006), and are commonly used as topical analgesics for many painful clinical conditions (Kaale *et al*., 2002).

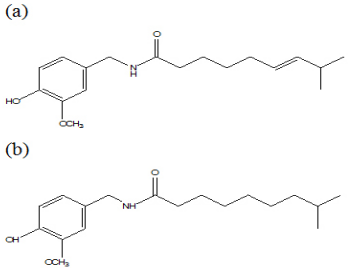


Figure 1. Chemical structure of (a) capsaicin and (b) dihydrocapsaicin

The quantitative analysis of capsaicinoids in chilli pepper, topical cream (Kaale *et al.*, 2002), self-defense weapons (Reilly *et al*., 2001), aerosol defense sprays (Spicer et al., 2005) and pepper sauces (Pena-Alvarez, 2009) has been interest for many reasons.

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Extraction techniques of capsaicinoids from hot chilli pepper have numerously been developed such as maceration (Titze *et al.*, 2002), Soxhlet (Korel *et al.*, 2002) and ultrasound-assisted extraction (Karnka *et al.*, 2002). The capsaicinoids extract were subjected to analyze by various techniques including spectrophotometry (Perucka and Oleszek, 2000) and thin layer chromatography (Materska and Perucka, 2005). In recent years, the popular techniques for the analysis of capsaicinoids are high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS).

Recently, the capsaicinoids have attracted the interest of many researchers because they show promise of being powerful antioxidants, protecting the human body from free radicals. In addition, one benefit of hot chilli is that they contain bioactive components such as polyphenol that can reduce oxidative stress and modulate harmful biological pathways, thereby reducing these chronic diseases. Various polyphenols have been reported to show radical scavenging activity (Sawa *et al.*, 1999) as well as an inhibiting α-amylase and α-glucosidase activities (Kim *et al.*, 2000).

This study was aimed to investigate both qualification and quantification of major and minor capsaicinoids in some varieties of hot chilli grown in Northeast of Thailand using a full scan mode of GC-MS. Additionally, α-amylase inhibitory activity of the chilli extracts was measured by reducing sugar method.

## Methods and Materials

*Chemicals*

All chemicals used were of analytical grade including α-amylase from Bacillus subtilis (51.5 U $mg^{-1}$) (Fluka, Switzerland), capsaicin and dihydrocapsaicin (Sigma, USA), 3,5-dinitrosalicylic acid (Fluka, China), D(+)-maltose monohydrate (Acros Organics, USA), potassium sodium tartrate (Carlo Erba, Italy), dimethylsulfoxide (Lab Scan, Thailand), methanol both analytical and HPLC grade (Lab Scan, Thailand) and sodium chloride (Analar, England). Tapioca power was of commercial grade, purchased from local market in Khon Kaen, Thailand.

*Instruments*

Determination of major and minor capsaicinoids in hot chilli samples was performed by GC-MS. Its system was controlled by Xcalibur software version 1.3, which compose of Trace GC chromatograph Model K073(0)910 (Italy) and Finnigan Polaris Q mass spectrometer (USA). An analytical column, ZB-5 capillary column coated with 5% diphenyl and 95% dimethylpolysiloxane (30 m × 0.25 mm, 0.25 µm film thickness), Phenomenex (USA) was employed. The inhibition of α-amylase was obtained by UV-Visible spectrophotometer (Spectronic 15, Thermo Scientific, USA) equipped with a 1-cm quartz cell.

A low pressure rotary evaporator, model R-200, Buchi (Switzerland) was used to reduce the solvent giving the crude residue. Hot plate stirrer, model MR 3001, Heidolph (Germany) and centrifuge, model EBA 20, Hettich Zentrifugen (Germany) were used for sample extraction. Vortex mixer, model G-560E (Scientific Industries Inc., (USA) and water bath, model Isotemp 228, Fisher Scientific (UK) were used to investigate the α-amylase inhibitory activity. The C18 cartridges (500 mg in 4 $cm^3$) were obtained from Alltech (USA). A Pipetman P20-1000 (Pipetman, France) micropipette and membrane filter, 0.45 µm (Whatman International, UK) were used.

*Plant samples*

Hot chilli samples were obtained from Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. Seven varieties of the chilli samples with their local breeding varieties belong to the same species, Capsicum annuum. L., include "Jindanil 80" (S1), "Numkaewtong 80" (S2), "Num Mordindang" (S3), "Superhot" (S4), "Yodson Khem 80" (S5), "Yodson Korat" (S6) and "Yodson Mordindang" (S7). The chilli fruit was dried in an oven at 60°C for 48 h and ground by a kitchen grinder (Moulinex Optiblend 2000, France) to pass a 30-mesh sieve. The ground chilli samples in sealed plastic bag were stored in a desiccator before use (Contreras-Padilla and Yahai, 1998).

*Sample preparation*

Extraction of capsaicinoids from hot chilli samples was carried out. This procedure consisted of two main steps: magnetic stirring extraction (MSE) and followed by solid-phase extraction (SPE). Two grams of the chilli sample were macerated using magnetic stirring with 20 mL of methanol at 60°C for 2 h. The extract solution was centrifuged at 5,000 rpm for 5 min to remove the residue of the plant sample and then filtered through a Whatman No. 42 filter paper. The solvent was evaporated to dryness using a rotary evaporator, and a crude extract was obtained. The chilli extract was re-dissolved with methanol to make a final volume of 5 mL and then passed through a C18 solid phase cartridge.

The solid-phase cartridge was conditioned with 2

mL of methanol and followed by 2 mL of deionized water. 400 µL of the extract solution was diluted with 600 µL deionized water and injected into the conditioned C18 cartridge. The cartridge was then rinsed with 2 mL of deionized water. Finally, the capsaicinoids were eluted with 2 mL methanol for two times and adjusted the final volume to 10 mL. The extraction solution was injected into the GC-MS after filtered through a 0.45 µm nylon filter membrane.

*Qualitative analysis of capsaicinoids*

The separation and characterization of the capsaicinoids extract were carried out by GC-MS. The initial oven temperature was 150°C and the final temperature 280°C. The temperature of transfer line was 275°C. Helium was used as carrier gas at a flow rate of 1.0 mL $min^{-1}$. The injection volume was 1.0 µL using splitless injection technique. The ionization of capsaicinoids can be performed by EI mode. The temperature program profile used in this study was optimized as following. The initial GC column temperature was 150°C, then programmed from 150 to 250°C (with ramp rate of 20°C $min^{-1}$) and up to the final temperature 280°C (with ramp rate of 2°C $min^{-1}$) and held for 2 min.

*Quantitative analysis of capsaicinoids*

The stock solution (1,000 µg $mL^{-1}$) of capsaicin and dihydrocapsaicin were prepared by dissolving in methanol. Working standard solutions were prepared by appropriate dilution of the stock solution with methanol. The major and minor components of the capsaicinoids were analyzed using a full scan MS.

*Method validation of GC-MS*

Linearity was investigated from calibration plots which were studied by analysis of standard mixtures ranged from 10 to 100 mg $L^{-1}$ of capsaicin and dihydrocapsaicin. All standard mixtures were prepared by dilution of the stock standard solution. Limit of detection (LOD) and limit of quantitation (LOQ) were also investigated by a linear regression. Linear ordinary least-squares regression parameters were calculated based on the analysis of ten replicates of the capsaicinoids at five different concentration levels. The precision of the proposed method was presented as the repeatability and reproducibility of retention time and peak area. For intra-day precision, the standard mixtures of capsaicin and dihydrocapsaicin (50 µg $mL^{-1}$) were analyzed in five replicates within a day. For inter-day precision, the standard mixtures of capsaicin and dihydrocapsaicin (50 µg $mL^{-1}$) were analyzed in five replicates for three different consecutive days.

*Determination of α-amylase inhibition activity*

There are several methods for an investigation of α-amylase activity (Hagberg, 1951; Park and Wang, 1991, Tundis *et al.*, 2007; Menichini *et al.*, 2009). In this work, the α-amylase activity was determined by reducing sugar method. A 0.1 M sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 was prepared by dissolving 2.76 g of potassium dihydrogen phosphate and 0.268 g of sodium hydroxide in 1000 mL of deionized water and adjusted to pH 6.9 with 2 M sodium hydroxide. A 0.25% (w/v) starch solution was prepared by dissolving 0.125 g of tapioca starch in 50 mL of 20 mM sodium phosphate buffer (pH 6.9 plus 6.7 mM sodium chloride). To facilitate the solubility by heating the starch solution in a glass beaker, directly on a hot plate stirrer using constant stirring, and bring to a boil and maintain the solution at this temperature for 15 min.

Sodium potassium tartrate solution was prepared by dissolving 12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH. 96 mM 3,5-dinitrosalicylic acid solution was prepared by dissolving 0.4381 g of 3,5-dinitrosalicylic acid in 20 mL deionized water. The color reagent solution was prepared by mixing both the reagents prepared, and dilute to 40 mL with deionized water. The solution should be stored in an amber bottle at room temperature and stable for six months. A stock standard solution (1,000 mg $L^{-1}$) of maltose was prepared by dissolving 0.1053 g of D(+)-maltose monohydrate in 100 mL deionized water. A stock standard solution (10 U $mL^{-1}$) of α-amylase enzyme solution was prepared by dissolving 1.946 mg of α-amylase enzyme in 10 mL of cold sodium phosphate buffer solution.

Capsaicinoids were then extracted from hot chilli samples as described above. The crude extract was dissolved with dimethylsulfoxide (DMSO) to make a final volume of 5 mL. The α-amylase inhibition assay was adapted from a method previously described (Tundis *et al*., 2007; Menichini *et al*., 2009). Briefly, a total of 500 µL of the extract and 500 µL of 20 mM sodium phosphate buffer (pH 6.9 plus 6.7 mM NaCl) containing α-amylase solution (1 U $mL^{-1}$) were incubated at 37°C for 5 min. After incubation, 500 µL of 0.25% (w/v) tapioca starch solution in the phosphate buffer was added, and the mixture re-incubated for 5 min. The reaction was stopped with 500 µL of 3,5-dinitrosalicylic acid solution (DNS). The reaction tubes were then incubated in a boiling water bath for 5 min, and then cooled to room temperature. The reaction mixture was then diluted after adding distilled water to make a final volume of 5 mL. The generation of maltose was quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-

5-nitrosalicylic acid, the product being detectable at 540 nm. The α-amylase inhibition was expressed as percentage of inhibition and calculated by the following equation:

$$\%\,\text{Inhibition} = 100 - \left( \frac{[\text{maltose}]_{test}}{[\text{maltose}]_{control}} \times 100 \right)$$

Where $[\text{maltose}]_{test}$ is the concentration of maltose in the presence of capsicum fruit extract, and $[\text{maltose}]_{control}$ is the concentration of maltose in the control reaction at 540 nm (containing all reagents except the test compound).

## Results and Discussion

*Qualitative analysis of major and minor capsaicinoids*

In hot chilli samples, nine components among major and minor capsaicinoids could be analyzed by GC-MS. The capsaicinoids including nornordihydrocapsaicin, nordihydro-capsaicin, N-vanillyl-nonanamide, capsaicin, dihydrocapsaicin, homocapsaicin, N-vanillyl-decanamide, homodihydrocapsaicin isomer I and II were obtained. The ion chromatograms of the chilli extracted from Jindanil 80 (S1, Figure 2), Numkaewtong 80 (S2), Num Mordindang (S3), Superhot (S4, Figure 3), Yodson Khem 80 (S5), Yodson Korat (S6) and Yodson Mordindang (S7) are comparatively demonstrated, and their data (retention time, mass characteristics and its fragment ions) are summarized in Table 1.

According to literature, both capsaicin and dihydrocapsaicin were proved to be the predominant capsaicinoids of the chilli extracts (peak No. 4 and peak No. 5 shown in Figure 2 and Figure 3). The identification of capsaicin and dihydrocapsaicin was based on the comparison of retention time and mass spectrum (MS) with the standard substances available. Furthermore, the fragments of m/z at 305 (compound 4) and 307 (compound 5) in the MS experiment produced the vanillyl moiety (m/z 137) as the base peak, resulting from cleavage of the aromatic ring structure (Schweiggert *et al*., 2006).

EI of the $M^+$ ions of compound 7 yielded the main product ion at m/z 137, corresponding to the loss of the acyl chain, and further protonated ions at m/z 182, 195 and 165 supporting their assignment for homocapsaicin (Table 1). Beside dihydrocapsaicin (peak No. 5), four representatives of the dihydrocapsaicin group were detected and showed a similar fragmentations of compound 1, 2, 3 and 6 led to the formation of the predominant ion at m/z 137 and further product ions at m/z 144 and were tentatively identified as nornordihydrocapsaicin,

Table 1. Retention time and characteristic mass fragments of the capsaicinoids extracted from hot chilli samples

| Peak No. | Type of capsaicinoids | $t_R$ (min) | $[M^+]$ m/z | Fragment ion (m/z) |
|---|---|---|---|---|
| 1 | Nornordihydrocapsaicin | 8.40 | 279 | 115, 143, 193 |
| 2 | Nordihydrocapsaicin | 9.06 | 293 | 122, 137, 151, 152, 195 |
| 3 | *N*-vanillyl-nonanamide | 9.51 | 293 | 121, 144, 151, 178 |
| 4 | Capsaicin | 10.03 | 305 | 133, 137, 152, 168, 195 |
| 5 | Dihydrocapsaicin | 10.31 | 307 | 122, 137, 151, 152, 178, 195 |
| 6 | *N*-vanillyl-decanamide | 10.82 | 307 | 110, 137, 151, 152, 156, 178, 195 |
| 7 | Homocapsaicin | 11.46 | 319 | 121, 137, 147, 165, 182 |
| 8 | Homodihydrocapsaicin isomer I | 11.75 | 321 | 137, 151, 152, 195 |
| 9 | Homodihydrocapsaicin isomer II | 11.91 | 321 | 136, 151, 153, 178, 195 |

Table 2. Repeatability and reproducibility of both major capsaicinoids

| Capsaicinoids | Repeatability ($n$ = 5) | | | | Reproducibility ($n$ = 3×5) | | | |
|---|---|---|---|---|---|---|---|---|
| | $t_R$ (min) | | Peak area | | $t_R$ (min) | | Peak area | |
| | Mean | %RSD | Mean | %RSD | Mean | %RSD | Mean | %RSD |
| Capsaicin | 10.04 | 0.06 | 173373 | 5.05 | 10.05 | 0.07 | 1716217 | 6.77 |
| Dihydrocapsaicin | 10.34 | 0.11 | 2104183 | 3.65 | 10.34 | 0.14 | 2081742 | 4.50 |

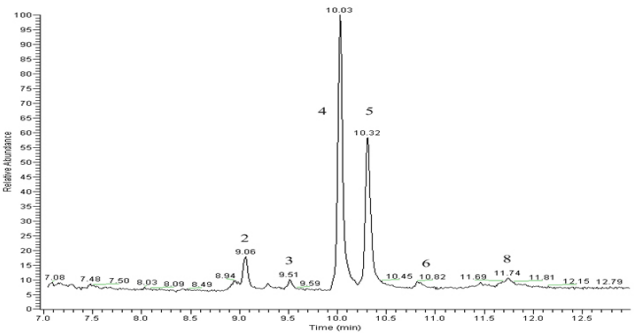


Figure 2. Ion chromatogram of (2) nordihydrocapsaicin, (3) N-vanillyl-nonanamide, (4) capsaicin, (5) dihydrocapsaicin, (6) N-vanillyl-decanamide and (8) homodihydrocapsaicin isomer I in "Jindanil 80" chilli sample (S1)

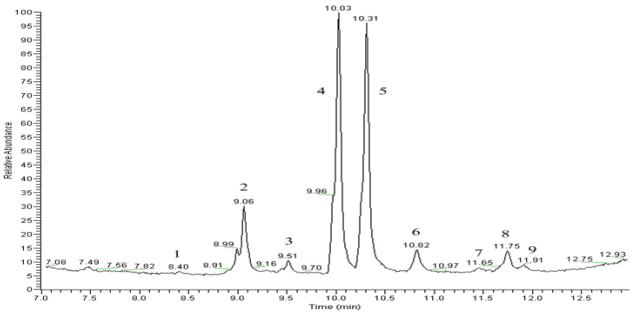


Figure 3. Ion chromatogram of (1) nornordihydrocapsaicin, (2) nordihydro-capsaicin, (3) N-vanillyl-nonanamide, (4) capsaicin, (5) dihydrocapsaicin, (6) N-vanillyl-decanamide, (7) homocapsaicin, (8) homo-dihydrocapsaicin isomer I and (9) homodihydrocapsaicin isomer II in "Superhot" chilli sample (S4)

nordihdrocapsaicin, N-vanillyl-nonanamide and N-vanillyl-decanamide, respectively. Compounds 8 and 9 exhibited $M^+$ ions at m/z 321 and were characterized as homodihydrocapsaicin isomer I and II, mainly based on the presence of the fragments at m/z 137 and 195, including the vanillyl and acyl moiety (Schweiggert *et al*., 2006).

*Quantitative analysis of major and minor capsaicinoids*

In order to evaluate the potential of the present method for quantitative uses, linearity, limit of detection, limit of quantification and precision

Table 3. The contents of nine components of the capsaicinoids found in chilli samples expressed as dihydrocapsaicin equivalent (µg/g DW) and their relative amount ratio (%)*

| Sample | Nornordihydrocapsaicin | Nordihydrocapsaicin | *N*-vanillyl-nonanamide | Capsaicin | Dihydrocapsaicin | *N*-vanillyl-decanamide | Homocapsaicin | Homodihydrocapsaicin isomer I | Homodihydrocapsaicin isomer II | Total Capsaicinoids |
|---|---|---|---|---|---|---|---|---|---|---|
| S1 | - | 202.0 ± 1.2 (3.6%) | 67.2 ±1.0 (1.2%) | 3363.4 ±3.0 (60.1%) | 1858.0 ±2.9 (33.2%) | 53.8 ±0.7 (1.0%) | - | 53.8 ±0.5 (1.0%) | - | 5598.2 (100.0%) |
| S2 | - | 121.5 ±1.2 (3.0%) | 20.1 ±1.6 (0.5%) | 2159.7 ±1.7 (52.6%) | 1804.9 ±1.0 (44.0%) | - | - | - | - | 4106.2 (100.0%) |
| S3 | - | 148.9 ±1.7 (3.1%) | 37.5 ±3.0 (0.8%) | 2924.4 ±4.2 (60.1%) | 1735.6 ±3.8 (35.6%) | - | - | 18.0 ±0.2 (0.4%) | - | 4870.2 (100.0%) |
| S4 | 9.7 ± 0.2 (0.1%) | 448.7 ±4.4 (5.9%) | 72.1 ±2.4 (0.9%) | 3462.9 ±6.9 (45.1%) | 3308.7 ±6.5 (43.1%) | 177.2 ±2.5 (2.3%) | 20.7 ±0.2 (0.3%) | 141.1 ±4.4 (1.8%) | 30.7 ±4.9 (0.4%) | 7671.8 (100.0%) |
| S5 | - | 157.0 ±0.3 (3.5%) | 54.1 ±0.1 (1.2%) | 2362.2 ±0.6 (52.3%) | 1902.5 ±0.1 (42.1%) | - | - | 42.0 ±0.1 (0.9%) | - | 4517.8 (100.0%) |
| S6 | - | 124.6 ±1.3 (3.4%) | 25.7 ±1.5 (0.7%) | 2250.9 ±1.8 (60.5%) | 1291.3 ±1.8 (34.7%) | - | - | 25.7±0.2 (0.7%) | - | 3718.2 (100.0%) |
| S7 | - | 202.5 ±2.4 (3.8%) | 43.6 ±1.8 (0.8%) | 2942.0 ±3.0 (55.4%) | 2050.1 ±2.9 (38.6%) | 29.2 ±1.9 (0.6%) | - | 48.4 ±1.7 (0.9%) | - | 5315.8 (100.0%) |

*Based on the normalized value (100.0%) of total capsaicinoids.
Mean ±%RSD, n = 3

Table 4. Total capsaicinoids content, Scoville heat unit (SHU) and their inhibition of α-amylase activity of the chilli samples

| Sample | Total capsaicinoids* (µg DC/g DW) | Scoville heat unit (SHU) | α-amylase activity inhibition (%)** |
|---|---|---|---|
| Jindanil 80 (S1) | 5598.2 | 84,000 | 19.8 ±2.0 |
| Numkaewtong 80 (S2) | 4106.2 | 61,600 | 21.6 ±3.7 |
| Num Mordindang (S3) | 4870.2 | 73,100 | 32.5 ±1.4 |
| Superhot (S4) | 7671.8 | 115,100 | 15.5 ±1.5 |
| Yodson Khem 80 (S5) | 4517.8 | 67,800 | 16.5 ±3.2 |
| Yodson Korat (S6) | 3718.2 | 55,800 | 32.1 ±1.1 |
| Yodson Mordindang (S7) | 5315.8 | 79,800 | 29.4 ±1.0 |

*Calculated as 1 µg/g of total capsaicinoids equivalent to 15 SHU.
**Mean ± %RSD; n = 3

were investigated. The quantitative features of the proposed method were studied under the optimum conditions. The linearity range for the capsaicin and dihydrocapsaicin was 10.0 – 100.0 µg $mL^{-1}$. Excellent linearity was obtained between peak area (y) and the corresponding concentration (x) on the standard curve of the two capsaicinoids: capsaicin, y = 58,094x – 798,067 with $r^2$ = 0.9859, and dihydrocapsaicin, y = 62,771x – 773,250 with $r^2$ = 0.9928. The coefficient ($r^2$) values were higher than 0.98. Both limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the analysis of ten replicates of the capsaicinoids at five different concentration levels. The LOD values were found to be 1.1 and 1.5 µg $mL^{-1}$ for capsaicin and dihydrocapsaicin, respectively, while those of LOQ were 3.6 and 5.0 µg $mL^{-1}$ for capsaicin and dihydrocapsaicin, respectively.

The precision of the proposed method was presented as relative standard deviation (RSD) of retention time and peak area. The repeatability (intra-day precision) is the variability of the measurements obtained by one person while measuring the same item repeatedly. This is also known as the inherent precision of the measurement equipment. The repeatability was deduced from five replicates within a day (n = 5). The RSD of retention time was lower than 0.2%, ranging from 0.06 – 0.11% and RSD of peak area was lower than 6.0%, ranging from 3.65 – 5.05% (Table 2).

The reproducibility (inter-day) is the variability of the measurement system. Mathematically, it is the variability of the average values obtained by several operators while measuring the same item. The reproducibility was investigated from the experiments in 3 consecutive days (n = 3 x 5). The RSD of retention time was lower than 0.2%, ranging from 0.07 – 0.14% and RSD of peak area was lower than 7.0%, ranging from 4.50 – 6.77% (Table 2). The repeatability and reproducibility of the retention time and peak area for the capsaicinoids were in good precision which their RSDs.

GC-MS technique was used to quantify nine components of the capsaicinoids in the chilli extract. The contents of capsaicinoids were determined based on the standard curve of dihydrocapsaicin equivalent (µg DC). The amounts of total capsaicinoids in seven varieties of the chilli samples were found to be 3718.2 – 7671.8 µg DC $g^{-1}$ DW, as shown in Table 3. The relationship between total contents of capsaicinoids (µg DC $g^{-1}$ DW) and SHU rating was approximately 15 SHU equal to 1 µg $g^{-1}$ of total capsaicinoids (Mathur *et al*., 2000). The results indicated that the "Superhot" (S4) sample gave the highest SHU according to total contents of the capsaicinoids (Table 4).

*α-Amylase inhibitory activity of the chilli extract*

Reducing sugar, the product of the digestion of starch, was reacted with 3,5-dinitrosalicylic acid to form 3-amino,5-ninitrosalicylic acid which absorbs light strongly at 540 nm. The effect of various amounts of the extract sample on an inhibition of α-amylase was studied and their result was expressed as the percentage inhibition of α-amylase activity. The percentage of α-amylase inhibition was increased with increasing amounts of the extract sample. However, the extract sample used at 20 µL showed constantly the percentage of α-amylase inhibition.

Seven varieties of the hot chilli were used to investigate the potential reduction of α-amylase activity, since this enzyme is known as one of key enzymes in the human digestive system to degrade

starch to monosaccharide and cause the rise in blood glucose (Hirsh *et al*., 1997). The results of α-amylase inhibitory activity of the chilli crude extract are also shown in Table 4. The percentages of α-amylase inhibition were obtained in the range of 15.5 – 32.5 % and "Num Mordindang" chilli sample showed the highest inhibitory activity. From the results, it is reasonably suggested that the capsaicinoids extract of these hot chilli samples seem to exhibit a wide range of the antidiabetic property.

## Conclusions

In hot chilli sample, nine components among major and minor capsaicinoids could be analyzed by GC-MS. The hot chilli sample "Superhot" contained all nine components of the capsaicinoids. These major and minor capsaicinoids were also quantified using GC-MS. Analytical performance and the method validation was evaluated. In real sample analysis, the capsaicinoids extracted from seven varieties of the chilli samples were investigated. All nine components of the capsaicinoids were found in "Superhot" chilli sample. Total contents of the capsaicinods in these samples were found in the range of 3718.2 – 7671.8 μg DC $g^{-1}$ DW. Their SHU were ranged of 55,800 – 115,100. The highest content of total capsaicinoids was found in "Superhot" chilli sample. In addition, the α-amylase inhibition activity was ranged of 15.5 – 32.5%, and both "Num Mordindang" and "Yodson Korat" chilli samples gave the highest inhibition activity.

## Acknowledgements

The authors gratefully acknowledge the financial supports from the Center of Excellence for Innovation in Chemistry (PERCH-CIC PERDO), the Office of the Higher Education Commission, Thailand, and the Hitachi Scholarship Foundation, Tokyo, Japan.

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