Determination of Major and Minor Capsaicinoids by GC-MS and the Inhibition of Alpha-Amylase Activity of the Hot Chilli Extracts

การหาองค์ประกอบหลักและองค์ประกอบรองของแคปไซซินอยด์โดยเทคนิคแก๊สโครมาโทกราฟี - แมสสเปกโทรเมทรี และการยับยั้งเอนไซม์แอลฟาอะไมเลสด้วยสารสกัดจากพริก

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ABSTRACT

Capsaicinoids were extracted from hot chilli (*Capsicum annuum* L.) and quantitatively determined by gas chromatography-mass spectrometry (GC-MS). Nine components of the capsaicinoids were detected, two major and seven minor capsaicinoids including capsaicin, dihydrocapsaicin, nornordihydrocapsaicin, nordihydrocapsaicin, *N*-vanillyl-nonanamide, *N*-vanillyl-decanamide, homocapsaicin, homodihydrocapsaicin isomer I and homodihydrocapsaicin isomer II. The antidiabetic property of the capsaicinoids extract was evaluated using the inhibition effect of α-amylase activity. In this study, five varieties of Thai hot chilli samples were investigated for the inhibitory activity. The results indicated that percentages of the enzyme inhibition were found in the range of 15.52-32.46%.

บทคัดย่อ

งานวิจัยนี้ ได้วิเคราะห์แคปไซซินอยด์ในตัวอย่างพริก ด้วยเทคนิคแก๊สโครมาโทกราฟี-แมสสเปกโทรเมทรี สามารถพิสูจน์เอกลักษณ์สารในกลุ่มแคปไซซินอยด์ได้ทั้งหมด 9 ชนิด ซึ่งประกอบด้วยองค์ประกอบหลัก 2 ชนิด และองค์ประกอบรอง 7 ชนิด ได้แก่ แคปไซซิน ไดไฮโดรแคปไซซิน นอร์นอร์ไดไฮโดรแคปไซซิน นอร์ไดไฮโดรแคปไซซิน เอ็น-วานิลลิลนอนานาไมด์ เอ็น-วานิลลิลเดคานาไมด์ โฮโมแคปไซซิน โฮโมไดไฮโดรแคปไซซิน ไอโซเมอร์ I และ โฮโมไดไฮโดรแคปไซซิน ไอโซเมอร์ II ส่วนการศึกษาคุณสมบัติในการยับยั้งการทำงานของเอนไซม์แอลฟาอะไมเลส ด้วยสารสกัดแคปไซซินอยด์ในพริก 5 ชนิดได้ผลการยับยั้งในช่วงร้อยละ 15.52-32.46

Key Words: Capsaicinoids, Alpha-amylase inhibition, GC-MS

คำสำคัญ: แคปไซซินอยด์ การยับยั้งเอนไซม์แอลฟาอะไมเลส แก๊สโครมาโทกราฟี-แมสสเปกโทรเมทรี

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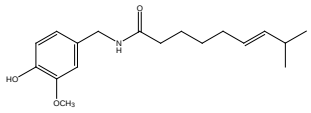
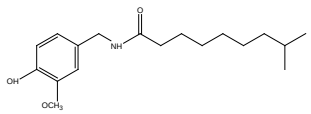
Introduction

Hot chillies are popular spices in many parts of the world. The properties of color, aroma, flavor and pungency were presented by these chilli accounts for their extensive usage. They are a rich source of valuable phytochemicals such as capsaicinoids. The capsaicinoids are the organic compounds responsible for hot, spicy flavor presented in many varieties of the chillies. It is composed of a vanillylamide moiety and an acyl chain containing 8-13 carbon atoms as shown in Fig. 1 (Schweiggert et al., 2006). Two major capsaicinoids present in the most varieties of the hot chilli are capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-*N*-vanillylnonanamide). In addition, other minor capsaicinoids are also found in the hot chilli, including nordihydrocapsaicin, norcapsaicin, homocapsaicin, homodihydrocapsaicin, nornorcapsaicin, nornornor-capsaicin, and nonivamide (Barbero et al., 2008).



(A)



(B)

Fig. 1 Chemical structure of (A) capsaicin and (B) dihydrocapsaicin

Furthermore, one benefit of hot chilli is that it contains bioactive components such as polyphenols that can reduce oxidative stress and modulate harmful biological pathways. The polyphenol compounds also offer an attractive strategy to control postprandial hyperglycemia, assist in weight management, and in the management of cardiovascular disease with minimal side effects (Kwon et al., 2006; Jaiboon et al., 2010; Oben et al., 2010). Various polyphenols have been reported to show an inhibiting α-amylase and α-glucosidase activities (Kim et al., 2000).

Therefore, the purpose of this research was to identify the major and minor capsaiciniods in the hot chilli samples by using full scan mode of GC-MS. In addition, the α-amylase inhibitory activity is also investigated concerning on an antidiabetic property of the crude extract.

Materials and methods

Plant materials

Hot chilli samples were obtained from Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University. Each group of hot chilli samples was dried in an oven at 60°C for 48 hours and ground by a kitchen grinder (Philips HR2067, the Netherlands) to pass a 30-mesh sieve. The following so-called in local Thai name of these samples were used in this study: "Jindanil 80", "Num Mordindang", "Superhot", "Yodson Korat" and "Yodson Mordindang". The ground samples in sealed plastic bag were stored in a desiccator before use.

Preparation of capsaicinoids extract

Each sample (2.0 g) was macerated using magnetic stirring with 20 mL of methanol at 60 °C for 2 h. The extract solution was centrifuged at 5000 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 (R-200 Buchi, Switzerland), and the crude extract obtained was kept for further experiment (Juangsamoot et al., 2007).

Quanlitative analysis of capsaicinoids

The capsaicinoids extract was analysed by gas chromatography-mass spectrometry (GC-MS). GC-MS system was controlled by Xcalibur software version 1.3, which compose of Trace GC chromatograph, model K073(0)^{9}10 (Italy) and Finnigan Polaris Q mass spectrometer (USA). ZB-5 column (30 m x 0.25 mm, 0.25µm film thickness), Phenomenex (USA) was employed. The optimum GC conditions were as follow: the initial oven temperature was 150^{o}C, then programmed from 150 to 250^{o}C at 20^{o}C/min and up to the final temperature 280^{o}C at 2^{o}C/min hold for 2 min. The ionization of capsaicinoids can be performed by EI mode. The capsaicinoids extract was dissolved with methanol to make a final volume of 5.0 mL and then passed through a C18 solid phase cartridge. The extract solution was injected into the GC-MS after filtered through a 0.45 µm nylon filter membrane.

Alpha-amylase inhibition assay

The capsaicinoids extract was dissolved with DMSO to make a final volume of 5.0 mL. The 50-fold dilution of the capsaicinoids extract solution was used to measure the α-amylase activity inhibition. The α-amylase inhibition assay method was adapted from a method previously described (Tundis et al., 2007; Menichini et al., 2009). Briefly, a total of 500 µL of the capsaicinoids extract and 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 plus 0.006 M NaCl) containing α-amylase solution (1 unit/mL) were incubated at 37^{o}C for 5 min. After incubation, 500 µL of 0.25% tapioca starch solution in 0.02 M sodium phosphate buffer (pH 6.9 plus 0.006 M NaCl) 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:

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

Results and discussion

Quanlitative analysis of capsaicinoids by GC-MS

In hot chilli, nine components among major and minor capsaicinoids could be analyzed by GC-MS.

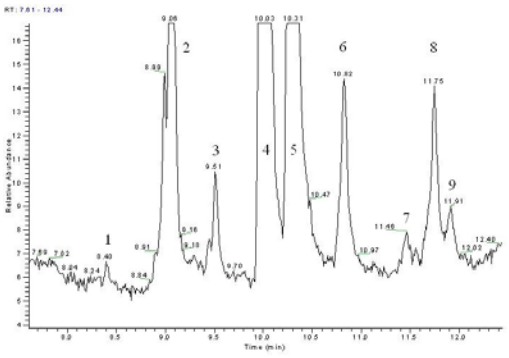


Fig. 2 Separation of major and minor capsaicinoids from hot chilli by GC-MS

According to literature, both capsaicin and dihydrocapsaicin were proved to be the predominant

capsaicinoids of the hot chilli extract (peaks 4 and 5 in Fig.2). The identification of capsaicin and dihydrocapsaicin was based on the comparison of retention time and mass spectrum 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 5), four representatives of the 'dihydrocapsaicin group' were detected and showed a similar fragmentation behavior as described for capsaicin. 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, nordihydrocapsaicin, *N*-valnillyl-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 fragments at m/z 137 and 195, indicating the vanillyl and acyl moiety (Schweiggert et al., 2006).

Alpha-amylase inhibition of hot chilli extract

Five varieties of hot chilli sample were used to investigate the potential reduction of diabetes risk. They were examined for α-amylase inhibitory 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).

Table 1 Characteristic fragments of capsaicinoids extracted from hot chilli

Peak	Capsaicinoids	RT min	$[M^+]$ m/z	Fragment ion (m/z)	Molecular form
1	Nornordihydro-capsaicin	8.40	279	115, 143, 193	$C_{16}H_{25}NO_3$
2	Nordihydrocapsai-cin	9.06	293	122, 137, 151, 152, 195	$C_{17}H_{27}NO_3$
3	*N*-vanillyl-nonanamide	9.51	293	121, 137, 144, 151, 178	$C_{17}H_{27}NO_3$
4	Capsaicin	10.03	305	133, 137, 152, 168, 195	$C_{18}H_{27}NO_3$
5	Dihydrocapsaicin	10.31	307	122, 137, 151, 152, 178, 195	$C_{18}H_{29}NO_3$
6	*N*-vanillyl-decanamide	10.82	307	137, 151, 152, 156, 178, 195	$C_{18}H_{29}NO_3$
7	Homocapsaicin	11.46	319	121, 137, 147, 165, 182, 195	$C_{19}H_{29}NO_3$
8	Homodihydrocapsa icin isomer I	11.75	321	137, 151, 152, 195	$C_{19}H_{31}NO_3$
9	Homodihydrocapsa icin isomer II	11.91	321	137,151, 153, 178, 195	$C_{19}H_{31}NO_3$

The results of α-amylase inhibition are shown in Table 2. As the so-called in Thai, "Num Mordindang" showed the highest activity (32.46%) and followed by "Yodson Korat" (32.09%), "Yodson Mordindang" (29.35%), "Jindanil 80" (19.83%), and "Superhot" (15.52), respectively. From the results, it is reasonably suggested that the capsaicionoids extract of these hot chillies have exhibited widely varying degree of the antidiabetic property.

Table 2 Inhibition (%) of alpha-amylase activity

Chilli variety[a]	α-amylase activity inhibition[b] (%)
Jindanil 80	19.83 ± 0.39
Num Mordindang	32.46 ± 0.35
Superhot	15.52 ± 0.23
Yodson Korat	32.09 ± 0.35
Yodson Mordindang	29.35 ± 0.28

[a] *Capsicum annuum*. L.

[b]Data are given as the mean ± standard deviation (n = 3).

Conclusion

In these hot chilli extracts, nine components of capsaicinoids were detected including two major components (capsaicin & dihydrocapsaicin) and seven minor ones (nornordihydrocapsaicin, nordihydro-capsaicin, *N*-vanillyl-nonanamide, *N*-vanillyl-decanamide, homocapsaicin, homodihydrocapsaicin isomer I and II). The spectrophotometric method for determination of α-amylase inhibition of the hot chilli extract was investigated. The average percentages of the enzyme inhibition were found in range of 15.52-32.46%, and the chilli "Num Mordindang" showed the highest inhibitory activity of α-amylase.

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