Hotness and Pungent Odour Profiles of Processed Dried Chilli (*Capsicum annuum* Linn. var. *Acuminatum Fingerh*.)

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ชื่อวิทยานิพนธ์ เค้าโครงความเผ็ดและกลิ่นฉุนของพริกแห้ง (*Capsicum annuum* Linn var. *Acuminatum Fingarh*.)
ผู้เขียน นางสาวนิจฉรา ทูลธรรม
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บทคัดย่อ

พริกชี้ฟ้าแห้ง (*Capsicum annuum* Linn var. *Acuminatum Fingarh*.) เป็นเครื่องปรุงอาหารที่รู้จักกันดีว่าให้คุณลักษณะทางประสาทสัมผัสด้านความเผ็ด กลิ่นฉุน และกลิ่นหอมในอาหารหลายชนิด ในงานวิจัยนี้ได้ผลิตตัวอย่างพริกแห้งจากพริกชี้ฟ้า โดยเลือกใช้กระบวนการทำแห้งที่แตกต่างกัน 3 วิธี คือ การทำแบบแห้งแช่เยือกแข็ง (FD) การทำแห้งด้วยลมร้อน (HD) และการทำแห้งแบบตากแดด (SD) เพื่อศึกษาผลของกระบวนการทำแห้งต่อคุณภาพทางกายภาพและเคมี ได้แก่ ปริมาณกรดแอสคอร์บิค กรดทั้งหมด สารให้ความเผ็ด (แคปไซซิน) และสารให้กลิ่นรสที่ระเหยได้ รวมถึงลักษณะทางประสาทสัมผัสของตัวอย่างพริกแห้งซึ่งประเมินโดยกลุ่มผู้ทดสอบฝึกฝน (n=15) นอกจากนี้ได้ประเมินขีดเริ่มรู้สึกจำ (Recognition threshold) ของความเผ็ดและกลิ่นฉุนของพริกแห้ง รวมถึงสารมาตรฐานที่ให้ความเผ็ดและกลิ่นฉุนโดยใช้กลุ่มผู้บริโภค 3 กลุ่ม คือ กลุ่มคนที่บริโภคอาหารเผ็ดน้อย (n=40) เผ็ดปานกลาง (n=40) และเผ็ดมาก (n=40) นอกจากนี้ได้สำรวจความชอบต่อความเข้มของลักษณะความเผ็ดและกลิ่นฉุนในกลุ่มผู้บริโภคดังกล่าวด้วย

งานวิจัยนี้สรุปได้ว่ากระบวนการทำแห้งที่แตกต่างกันมีผลต่อคุณภาพของตัวอย่างพริกแห้งที่รับรู้ได้โดยกลุ่มผู้ทดสอบฝึกฝน ได้แก่ สี ความฉุน และความเผ็ด ($P \leq 0.05$) อย่างไรก็ตาม เมื่อวิเคราะห์ปริมาณสารแคปไซซินของตัวอย่างพริกแห้งที่ผ่านการทำแห้งทั้ง 3 วิธี โดยเครื่องโครมาโทกราฟีของเหลวสมรรถนะสูง (High Performance Liquid Chromatography; HPLC) พบว่าปริมาณของสารแคปไซซินไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P>0.05$) และจากการวิเคราะห์สารให้กลิ่นรสที่ระเหยได้โดยเครื่องแก๊สโครมาโทกราฟี-แมสสเปกโทรเมทรี (*Gas chromatography-mass spectrometry*; GC-MS) พบว่า 1-penten-3-one (1P3O) คือสารหลักที่ระเหยได้ในตัวอย่างพริก และถูกรับรู้ได้ว่ามีกลิ่นฉุนโดยกลุ่มผู้ทดสอบฝึกฝน จากการวิเคราะห์ด้วยเทคนิค Partial Least Square regressions (PLS) พบว่าสารสำคัญที่ให้คุณลักษณะกลิ่นฉุน (1P3O) และความเผ็ด (แคปไซซิน) ในตัวอย่างพริกแห้งที่ระบุได้จากเครื่อง GC-MS เป็นปัจจัยที่มีผลต่อกลิ่นฉุนและความเผ็ดที่รับรู้ได้โดยกลุ่มผู้ทดสอบฝึกฝน

กลุ่มผู้ทดสอบฝึกฝนได้สร้างคำศัพท์เฉพาะจำนวน 12 คำ เพื่ออธิบายลักษณะทางประสาทสัมผัสของตัวอย่างพริกแห้ง และใช้มาตราหมายขนาด (Labelled Magnitude Scale; LMS)

ในการวิเคราะห์ด้านประสาทสัมผัสเชิงพรรณนาของตัวอย่าง ได้แก่ ตัวอย่างพริกบด และตัวอย่างพริกที่อยู่ในรูปของสารละลาย รวมถึงตัวอย่างสารละลายมาตรฐานของ 1P3O และแคปไซซิน พบว่าตัวอย่างพริกบดมีความเข้มของกลิ่นฉุนสูงกว่าตัวอย่างพริกที่อยู่ในรูปของสารละลาย ตัวอย่าง FD มีความเข้มของกลิ่นพริกสดและคุณลักษณะในกลุ่มความเผ็ดสูงกว่าตัวอย่าง HD และ SD ($P \leq 0.05$) นอกจากนี้ยังพบว่าตัวอย่าง FD และ HD มีความเข้มของลักษณะกลิ่นฉุนขึ้นจมูก กลิ่นฉุนแสบจมูก และความรู้สึกแสบร้อนในปากไม่แตกต่างกันอย่างมีนัยสำคัญ ($P > 0.05$) ในขณะที่ตัวอย่าง SD มีสีแดงคล้ำมากที่สุด และมีคุณลักษณะในกลุ่มความเผ็ดและกลิ่นฉุนที่น้อยที่สุด

ขีดเริ่มรู้สึกจำของกลิ่นฉุนและความเผ็ดของพริกแห้ง (ตัวอย่าง HD) สารละลายมาตรฐาน 1P3O และแคปไซซิน ที่ถูกประเมินโดยกลุ่มผู้บริโภคคนไทย 3 กลุ่ม โดยใช้วิธีการทดสอบบังคับเลือกหนึ่งในสาม (3-Alternative Forced Choice; 3-AFC) ด้วยการนำเสนอตัวอย่างที่ถูกเจือจางในช่วงความเข้มข้น 12 ระดับ จากต่ำไปสูง ขีดเริ่มรู้สึกจำของกลุ่มผู้บริโภคแต่ละกลุ่มถูกคำนวณด้วยวิธีการคำนวณแบบ 1) The Best Estimated Thresholds (BET; ASTM E697, 2004) และ 2) วิธีการ*วิเคราะห์การถดถอยโลจิสติก* (ASTM E1432, 2011) จากผลการทดลองพบว่ากลุ่มคนที่บริโภคเผ็ดมากมีขีดเริ่มรู้สึกจำของกลิ่นฉุนของพริก (5.88 กรัม/ลิตร) สารมาตรฐาน 1P3O (1.27 ไมโครกรัม/ลิตร) ความเผ็ดของพริก (17.19 กรัม/ลิตร) และสารมาตรฐานแคปไซซิน (11.75 มิลลิกรัม/ลิตร) มากที่สุดเมื่อเทียบกับกลุ่มคนที่บริโภคเผ็ดปานกลาง และเผ็ดน้อย นอกจากนี้ยังพบว่าผู้บริโภคทั้ง 3 กลุ่ม มีความชอบต่อความเผ็ดและกลิ่นฉุนแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P \leq 0.05$) โดยกลุ่มคนที่บริโภคเผ็ดน้อยชอบความเผ็ดและกลิ่นฉุนของพริกที่ระดับความเข้มอ่อน (0.58 และ 0.61 กรัม/ลิตร) ในขณะที่กลุ่มคนที่บริโภคเผ็ดปานกลางชอบความเผ็ดและกลิ่นฉุนของพริกที่มีความเข้มในระดับปานกลาง (2.23 และ 1.75 กรัม/ลิตร) และกลุ่มคนที่บริโภคเผ็ดมากชอบความเผ็ดและกลิ่นฉุนของพริกที่ระดับความเข้มสูง (7.19 และ 5.88 กรัม/ลิตร)

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ABSTRACT

Dried Chee fah chilli (*Capsicum annuum* Linn. var. *Acuminatum Fingarh*.) is a well known condiment. Its sensorial properties contribute to hotness, pungent odour, taste and aroma for many foods. In this research, freeze (FD), hot air (HD) or sun (SD)-dried chilli samples were produced and the effects of the drying methods were compared on the basis of their qualities. Physical and chemical qualities, such as ascorbic acid content, total acidity, capsaicin content and volatile flavour, as well as sensory characteristics of dried Chee fah chilli were evaluated by objective and subjective measurements. Also, recognition thresholds of pungent odour and hotness in dried chilli and standard solutions were investigated among light-, moderate- and heavy chilli-users, in addition to a hedonic test to explore the ranges of preferred concentrations of key sensory attributes.

The result from this research could be concluded that drying methods had a significant impact on perceived qualities of dried chilli samples, including colour, pungent odour and hotness ($P \leq 0.05$). However, the capsaicin contents of the samples from three drying methods, which were analysed by High Performance Liquid Chromatography (HPLC), showed no significant difference ($P > 0.05$). 1-penten-3-one (1P3O) was a key pungent odour compound of chilli samples identified by Gas Chromatography-Mass Spectrometry (GC-MS) and was perceived as pungent odour by the trained panellists. The major compounds representing pungent odour (1P3O) and hotness (capsaicin) characteristics in dried chilli were identified by trained panellists as well as GC-MS. Partial Least Square (PLS) regressions were applied on the data sets to specify the key characteristics of the samples.

Trained panellists (n=15) developed the sensory lexicon consisting of 12 sensory attributes. Labelled Magnitude Scale (LMS) was used in the sensory descriptive analysis of chilli samples in ground and solution forms, as well as standard compound solutions. The ground dried chilli samples were perceived to have higher pungent odour intensities when compared to those produced by the same drying methods in solution form. The intensities of fresh chilli odour and hotness-related attributes were found to be higher in FD than in HD and SD ($P \leq 0.05$). Interestingly, the attributes of raise-to-nasal pungent odour, stinging-pungent odour and oral sting were not perceived differently between FD and HD ($P > 0.05$). SD contained the darkest red colour and the least hotness and pungent odour. Whereas the trained panellists could not discriminate between the intensities of pungent odour of FD and HD samples, the highest content of 1P3O was found in FD by GC-MS

The recognition thresholds of pungent odour and hotness of dried chilli (HD sample) and 1P3O and capsaicin standard solutions were determined by three groups of Thai chilli-users. The identifying recognition thresholds of the two sensorial attributes were conducted by an ascending 3-Alternative Forced Choice (3-AFC) with 12 concentrations of sample dilutions in ranges. The group thresholds were calculated based on the Best Estimated Thresholds (BET; ASTM E697, 2004) and logistic regression approaches (ASTM E1432, 2011). Heavy chilli-users showed the highest recognition thresholds of the pungent odour of dried chilli (5.88 g/l) and 1P3O (1.27 µl/l), and of hotness of dried chilli (7.19 g/l) and capsaicin (11.75 mg/l). In relation to liking, light (n=40), moderate (n=40) and heavy (n=40) chilli-users were significantly different on hotness and pungent odour ($P \leq 0.05$). Each group of consumers liked hotness and pungent odour of dried chilli samples at middle levels of their group threshold brackets, which were mild for light chilli-users (0.58 and 0.61 g/l), average for moderate chilli-users (2.23 and 1.75 g/l), and strong for heavy chilli-users (7.19 and 5.88 g/l), respectively.

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ABBREVIATION

F = Fresh chilli sample
FD = Freeze Dried chilli sample
HD = Hot air Dried chilli sample
SD = Sun Dried chilli sample
SF = Solution of Fresh chilli sample
SFD = Solution of Freeze Dried chilli sample
SHD = Solution of Hot air Dried chilli sample
SSD = Solution of Sun Dried chilli sample
1P3O = 1-Penten-3-One
DRC = Dark Red Colour
BO = Burnt chilli Odour
FO = Fresh chilli Odour
RNO = Raise-to-Nasal pungent Odour
SPO = Sting Pungent Odour
WM = Warm in Mouth
WMS = Warm in Mouth after Spitting
OB = Oral Burn
OBS = Oral Burn after Spitting
OS = Oral Sting
OSS = Oral Sting after Spitting
TN = Tongue Numb
LMS = Labelled Magnitude Scale
3-AFC = 3-Alternative Forced-Choice
HPLC = High Performance Liquid Chromatography
GC-MS = Gas Chromatography-Mass Spectrometry
PCA = Principle Component Analysis
PLS = Partial Least Square Regression
SHU = Scoville Heat Units
GDA = Generic Descriptive Analysis
LLE = Liquid-Liquid Extraction
SPME = Solid Phase Microextraction

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Chee fah chilli (*Capsicum annuum* Linn. var. *Acuminatum Fingerh*.) has an unique in pungent odour and hotness, so it is widely used as an ingredient for flavour, pungency and colour enhancer. In general, dried chilli production is mostly used sun-, hot air- and freeze-drying methods. Among three drying methods, sun drying is a traditional process for drying chilli. However, it is difficult to control chilli quality (Prakash and Eipeson, 2003). On the other hand, hot air drying is an alternative process which has become more popular for drying chilli due to short drying time, uniform heating and more hygienic characteristics. It is widely used to produce dried chilli in industrial factory (Chung *et al*., 1992). Another drying method, freeze drying is the best process for retention dried chilli quality (Park and Kim, 2007), however it is the most expensive process for drying (Ratii, 2001). All drying methods, such as sun-, hot air-and freeze-drying had mainly affected on colour and ascorbic content of chilli (Márkus *et al*., 1999; Howard *et al.*, 1994; Daood *et al.,* 1996; Topuz and Ozdemir, 2004; Mínguez-Mosquera *et al*., 1994; Park and Kim, 2007). Likewise, the volatile flavour and hotness attributes of chilli can be destroyed during drying, but they still have limit identified. Capsaicin is the most prevalent capsaicinoids that is responsible for hotness attribute of chilli (Kobata *et al.*, 1998). The hotness attribute is described as hot, sharply, heat, bite, fiery, a burning sensation by taste reception and mouth burning (Eissa *et al*., 2007; Toontom, 2008). Among volatile flavour compounds in chilli, a pungent volatile compound (e.g. 1-penten-3-one) is responsible for a character impacting odour of chilli (Luning *et al.*, 1995; Van Ruth *et al*., 1995). The pungent odour attribute is a senses of smell by chilli, as described as strong odour, sharp pungent odour and pungent sensation by nasal perception (Tainter and Grenis, 2001; Toontom, 2008). During drying chilli, capsaicin was exposed to greater thermal and oxidative degradation, thus the drying temperature might be affected on the levels of capsaicin available in chilli

(Pordestimo *et al.*, 2004). Likewise, the volatile compounds of chilli can be decomposed during drying process (Luning *et al.*, 1995; Govindarajan, 1986; Venskutonis, 1997; Lin and Durance, 1998; Szumny *et al*., 2010). In addition, the changes in hotness and volatile flavour of dried chilli concentration may not meet the requirement of the consumers in terms of flavour attributes. Therefore, hotness and volatile flavour quality and consumer acceptance are important criteria to be use for selecting an appropriate drying process of chilli. The concentration of hotness and volatile compounds may be differently perceived by consumers. The intensity of oral burn and odour depended on consumers with frequent and non-frequent hot food users (Prescott and Stevenson, 1995b). Likely, volatile flavour and hotness intensities might affect on different magnitude of pungent odour and hotness sensation between chilli-users and non-chilli users (Reinbach *et al*., 2007). Therefore, thresholds measurement of the different groups of chilli-consumer is an importance to provide a guideline for applying the amount of chilli content in each food product for each group of chilli-consumers.

1.2 Review of Literature

1.2.1 Chee fah chilli

Chilli (*Capsicum* sp.) belongs to Solanaceae family (Mínguez-Mosquera and Hornero-Méndez, 1994) and is considered as vegetables (Bosland, 1996). The main components of chilli pod are pedicel, placenta, seeds and pericarp (Rajput and Parulekar, 1998) (Figure 1). Carotenoids and capsaicinoids are the most important groups of chemical composition in chilli. The carotenoids contribute to colour and nutritional value of chilli. Wheareas, capsaicinoids contribute to hotness characteristic of chilli (Bosland and Votava, 1999). The capsaicinoids are highly found in the chilli's placenta (1.79% dry basis). The outside skin and the seeds do not contain capsaiciniods (Govindarajan and Sathyanarayana, 1991).

Chilli is valued spices for their sensory attributes of hotness, pungent odour and colour. Importantly, its hotness and pungent odour are sensory attributes which enhance flavour in bland food (Reineccius and Reineccius, 2002). In Thailand, people consume chilli approximately 1 kg/year as hot appetizer and seasoning

(Cheiwchanwit, 2002). Chilli is the main ingredient for cooking in order to present the characterization of Thai cuisine, such as Thai soup and curries paste (Pisalong, 2002).

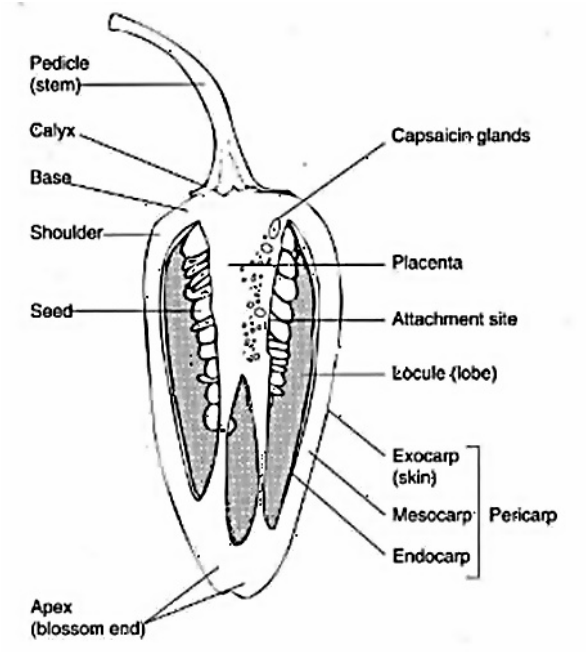


Figure 1 Components of chilli pod
Source: Rajput and Parulekar (1998)

Generally, the genus *Capsicum* is reported that about 20 species are distributed worldwide. The five major species of *Capsicum* cultivated are *Capsicum annuum*, *Capsicum frutescens*, *Capsicum chinense*, *Capsicum pubescens* and *Capsicum baccatum* (Csilléry, 2006). *Capsicum annuum* is more widely used than the other species by human in various continents (Mínguez-Mosquera and Hornero-Méndez, 1994; Bosland, 1996; Csilléry, 2006; Andrews, 1993). It is the most common species for dried spice production, because it produces large pod that are easier for harvesting and processing (Klieber, 2000).

In Thailand, *Capsicum annuum* is called sweet pepper (Prik Hwan or Prik Yaug), red chilli (Prik Dang) and Chee fah (Prik Chee fah or Cayenne pepper). Chee fah is admired to use for chilli paste (Yuenyongsawad, 2002).

Chee fah chilli (*Capsicum annuum* Linn. var. *Acuminatum Fingerh.*) is one of the two chilli types widely used in Thailand (Lertrat, 2007). Its pod has a finger shape with 4-6 inches in length. Its pod is upright in growth habit. The chilli is green

when unripe, changing principally to red when ripe. It can tolerate in most climates, especially in warm and dry climates. Chee fah chilli contains high vitamins A and C, but low calories and sodium. It also contains amounts of potassium, magnesium and folic acid. The chemical composition of Chee fah chilli is shown in Table 1. Hot level of Chee fah chilli is mild to medium with heat rate between 30,000-60,000 SHU (Wangcharoen and Morasuk, 2007). The main uses of Chee fah chilli is varied according to its pungency and colour, for example, use as a flavour enhancer, pungency enhancer and colour enhancer (Biacs *et al*., 1992; Raghavan, 2006).

Table 1 Chemical composition of 100 g Chee fah chilli

Chemical composition	Contents
Moisture (%)	84
Food energy (cal)	72
Protein (g)	2.8
Fat (g)	2.3
Carbohydrate (g)	10.1
Calcium (mg)	3
Phosphorus (mg)	18
Iron (mg)	1.3
Thiamine (mg)	0.16
Niacin (mg)	3.5
Ascorbic acid (mg)	168

Source: De (2003)

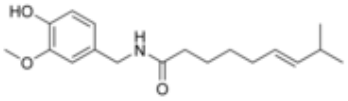
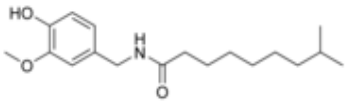
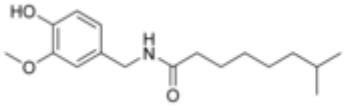
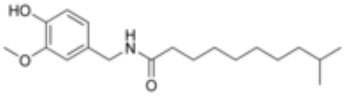
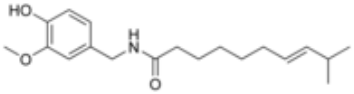
1.2.2 Main compounds responsible for chilli flavour characteristics

1.2.2.1 Hotness compounds

Hotness is the most typical attribute in chilli. Chilli hotness is a desirable attribute in many foods and increases the acceptance of the insipid basic nutrient foods in most of the world (Bosland, 1996; Al Othman *et al*., 2011). Hotness obtains from the secondary metabolism of alkaloid groups, namely capsaicinoids that are found only in the genus *Capsicum* (Kraikruan *et al*., 2008). The concentration of capsaicinoids in chilli typically ranges from 0.1 mg/g to 2.5 mg/g (Parrish, 1996).

Thomas *et al*. (1998) reported that chilli varieties, namely *Capsicum frutescens*, *Capsicum annuum* and *Capsicum chinense* contained 0.22-20 mg/g of capsaicinoids (dry basis). In Thai chilli, *Capsicum frutescens* and *Capsicum annuum* contained 0.76-3.76 mg/g of capsaicinoid (dry basis) (Kraikruan *et al*., 2008). Capsaicinoids are in a larger family of chemicals called the vanilloids, compounds that contain the vanillyl group by a condensation reaction between an aromatic moiety and a C9-C11 branch chain fatty acid (Garcés-Claver *et al*., 2006). Capsaicinoids are synthesized and accumulated preferentially in placenta rather than in pericarp and seeds (Cisneros-Pineda *et al*., 2007). The capsaicinoids in chilli are composed of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin (Andrews, 1995; Walsh and Hoot, 2001). The chemical structure and hotness level of all capsaiciniods are shown in Table 2 (Wall and Bosland, 1998). They are only difference in the presence of a carbon-carbon double bond (Harrison, 2001). The compounds, i.e. norcapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin are considered as minor capsaicinoids because of their relative low abundance in chilli. In addition, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin had lower hotness than capsaicin and dihydocapsaicin (Walsh and Hoot, 2001).

Table 2 Chemical structure and heat level of capsaiciniods

Capsaicinoid name	Typical relative amount	Scoville Heat Units (SHU)	Chemical structure
Capsaicin (C)	69%	16,000,000	
Dihydrocapsaicin (DHC)	22%	16,000,000	
Nordihydrocapsaicin (NDHC)	7%	9,100,000	
Homodihydrocapsaicin (HDHC)	1%	8,600,000	
Homocapsaicin (HC)	1%	8,600,000	

Source: Wall and Bosland (1998)

Both capsaicin and dihydrocapsaicin are constituted about 90% of the total capsaicinoids (Al Othman *et al*., 2011). The boiling point of capsaicin is 511.5^{o}C at 760 mmHg (Guidechem, 2011). While, dihydrocapsaicin is the second most prevalent one (Ben-Chaim *et al*., 2006) and its boiling point is 497.4^{o}C at 760 mmHg (Guidechem, 2011). Although, the hotness level of capsaicin equal to dihydrocapsaicin (Zewdie and Bosland, 2001), capsaicin is the most prevalent capsaicinoid (Ben-Chaim *et al*., 2006). Therefore, capsaicin content of chilli is one of the major parameters that determine its commercial quality (Ohnuki *et al*., 2001; Kawabata *et al*., 2006; Hachiya *et al*., 2007; Zhang *et al*., 2007).

Capsaicin (8-methyl-N-vanillyl-6-nonenamide: $C_{18}H_{27}NO_3$) is biosynthesized through the condensation of vanillylamine, a phenyl propanoid pathway intermediate, and fatty acid moieties in placental tissues of *Capsicum* fruits (Iwai *et al*., 1978) (Figure 2). Capsaicin is an odourless, flavourless and lipophilic substance. This compound is soluble in ethanol, acetone and fatty oils, but it is insoluble in cold water (Cordell and Araujo, 1993).

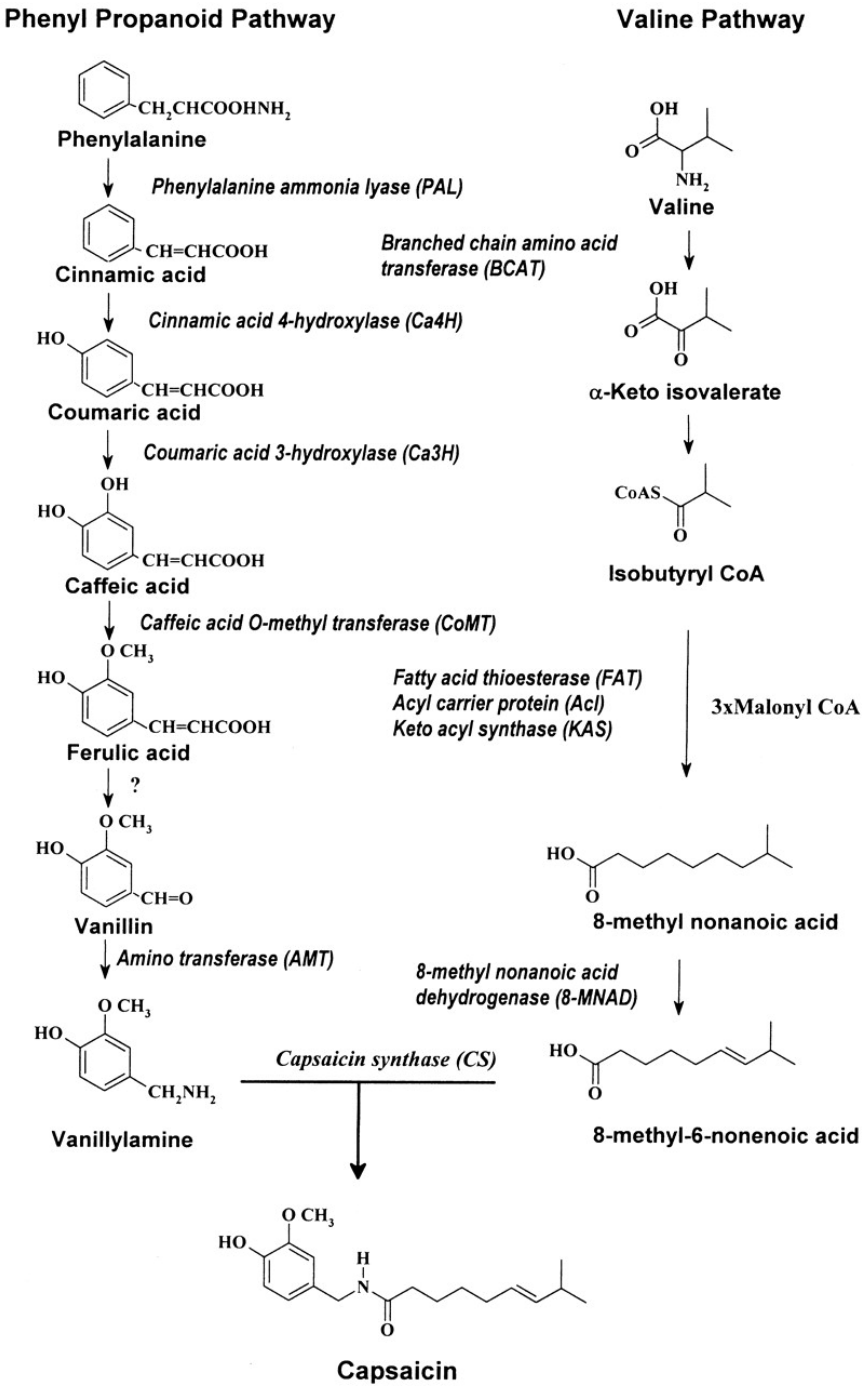


Figure 2 Capsaicin biosynthetic pathways

Source: Prasad *et al*. (2006)

Capsaicin is considered as an active principle which accounts for the pharmaceutical properties of chilli. It has been used as an analgesic against arthritis pain and inflammation (Deal *et al*., 1991). It has been reported to show anticancer

effect (Moore and Moore, 2003) and to be active against neurogenic inflammation (burning and stinging of hands, mouth and eyes) (Szolcsanyi, 2004). The latter property is the basis for the use of capsaicin in defensive chilli sprays. Capsaicin has also been reported to show protective effects against high cholesterol levels and obesity (Kempaiah *et al*., 2005). Capsaicin and other members of the capsaicinoids group produce a large number of physiological and pharmacological effects on the gastrointestinal tract, the cardiovascular and respiratory system as well as the sensory and thermoregulation systems. These effects result principally from the specific action of capsaicinoids on primary afferent neurons of the C-fiber type (Iida *et al*., 2003; Mózsik *et al*., 2005). This specific influence provides the rationale to treat some peripheral painful states, such as rheumatoid arthritis (Iida *et al*., 2003; Mózsik *et al*., 2005; Inoue *et al*., 2007; Derry, 2009; Backonja *et al*., 2010; Reyes-Escogido *et al*., 2011). However, high levels of capsaicin lead to negative health impacts. In a case-control study in Mexico-City which included 220 cases of gastric cancer and 752 controls randomly selected from the general population, chilli-users were at a 5.5-fold greater risk for gastric cancer than non-chilli users. Persons who rated themselves as heavy chilli-users were at an even higher 17-fold greater risk. However, when chilli consumption was measured as frequency per day, no significant dose to response relationship was observed (Lopez-Carrillo *et al*., 1994).

An average consumption of chilli was reported to be 2.5 g/person/day in India, 5 g/person/day in Thailand (Monsereenusorn, 1983) and 20 g/person per day in Mexico (Lopez-Carrillo *et al*., 1994). Assuming a content of capsaicin in these spices of about 1%, the daily intake of capsaicin in these countries has been estimated to 25–200 mg/person/day, which corresponds in the case of a person with 50 kg body weight (Council of Europe, 2001). The maximum daily intake of capsaicin in the U.S. and Europe from mild chillies and paprika was estimated to be roughly 0.025 mg/kg body weight/day (Govindarajan and Sathyanarayana, 1991), which is equivalent to 1.5 mg/person/day. Al Othman *et al*. (2011) estimated that the mean and maximum intakes of capsaicin from industrially prepared food products containing the recommended general limit of 5 μg/g, would be 0.77 and 2.64 mg/day, respectively.

The interest in capsaicin is mainly due to its hotness power. Most capsaicin researchers focused on the quantity changes (Garcés-Claver *et al*., 2006;

Sanatombi and Sharma, 2008), the pharmaceutical properties (Moore and Moore, 2003; Szolcsanyi, 2004; Kempaiah *et al*., 2005; Iida *et al*., 2003; Inoue *et al*., 2007; Derry, 2009; Backonja *et al*., 2010; Reyes-Escogido *et al*., 2011), sensitization and desensitization effects (Green, 1996; Karrer and Bartoshuk, 1995), detection threshold measurement (Lawless, 1989; Lawless *et al*., 2000) and taste marking effect (Baron and Penfield, 1996). However, limit information on capsaicin stability after drying process and capsaicin hotness recognition threshold in different chilli-users is studied.

1.2.2.2 Pungent odour compounds

The volatile oil in the chilli ranges from 0.1 to 2.6%, which impart to the characteristic odour and flavour of fresh chilli (Pruthi, 2003). The chilli has difference in volatile compounds which depends on the maturity stages (Mazida *et al*., 2005; Luning *et al.*, 1994a, b). The presence of various volatile compounds of chilli belongs to several chemical classes, i.e. phenols, aldehydes, acids, ketones, pyrazine, alcohols, ethers, nitrogen compounds, aromatic hydrocarbons, alkanes, esters and lactones (Chitwood *et al.,* 1983; Govindarajan, 1986; Luning *et al.,* 1995; Mateo *et al*., 1997).

Many researchers mentioned that alkylmethoxy-pyrazines are the character impact volatile compound of the genus *Capsicum* (Chitwood *et al.*, 1983; Mateo *et al.,* 1997; Cremer and Karl Eichner, 2000; Mazida *et al.*, 2005; Pino *et al*., 2007). Chitwood *et al.* (1983) suggested that 2-sec-butyl-3-methoxypyrazine and 2-methoxy-3-isobutylpyrazine are responsible for the frequent use of green descriptors in the odour descriptive analysis of *C. annuum* cultivar (Anaheim, Jalapeňo and Fresňo). Mazida *et al.* (2005) reported that 2-methoxy-3-isobutylpyrazine (grassy odour) was found to be significantly decreased during ripening, which agreed with the investigation of Chitwood *et al*. (1983) and Luning *et al.* (1994a, b). Chitwood *et al.* (1983) reported that 2-methoxy-3-isobutylpyrazine were recognized as being responsible for the characteristic aroma of the fresh chilli (bell pepper), which has an odour threshold level of 0.002 ppb in water. However, the 2-methoxy-3-isobutylpyrazine cannot be found in *C. annuum* (Spanish paprika) (Mateo *et al.,* 1997). Although, the character impact volatile compound of chilli is alkylmethoxy-pyrazines, it has not been reported that is responsible for pungent odour of chilli.

The pungent compound is explained by the basis of partial charges on atoms within the molecule. Its molecule has an electron-deficient region that is strongly attracted to an electron-rich site on the pungent receptor (Senese, 2011). There are a few researches which have been conducted on the presence of pungent odour compound in chilli (Luning *et al*., 1994a; Van Ruth *et al*., 1995). Luning *et al*. (1994a) reported that 10 of 12 trained panellists at a sniffing port on a gas chromatograph described 1-penten-3-one as pungent odour found in *C. annuum*. Likewise, Van Ruth *et al*. (1995) identified 47 volatile flavour components in *C. annuum* by using a sniffing port on a gas chromatograph with 12 trained panellists and found that 1-penten-3-one is a major pungent odour compound of *C. annuum*. Reineccius and Reineccius (2002) reported that the presence of 1-penten-3-one has an odour threshold level of 0.001 ppm in water. The chemical structure of 1-penten-3-one is shown in Figure 3.

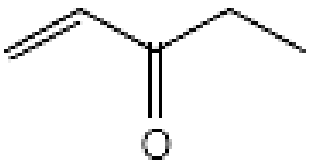


Figure 3 Chemical structure 1-penten-3-one
Source: JECFA (2002)

1.2.3 Chilli drying method

Drying is the classical method to preserve food for long periods and results in concentration of volatile flavour and nutrients (Nogueira *et al.*, 2005). Among drying methods, sun-, hot air- and freeze-drying is mostly used in dried chilli production. They were chosen to use by different reasons of products, such as familiar agricultural (for use in household), industrial and pharmaceutical products (Nogueira *et al.*, 2005; Chung *et al*., 1992; Irzyniec *et al.,* 1995).

1.2.3.1 Sun drying

Sun dried chilli is produced and commercialized by familiar agriculture. Although being a popular and very inexpensive method, the dried chilli products suffer undesirable fermentation and result reduction in the sales (Nogueira *et*

al., 2005). Sun drying of chilli normally takes about 7-20 days (depending on the weather conditions) to reduce the moisture content between 10 and 15% (Oberoi *et al.*, 2005; Hossain, 2003). However, sun drying cannot be controlled and can lead to dull colour formation in chilli (Prakash and Eipeson, 2003). Topuz and Ozdemir (2004) reported that sun drying leaded to a decrease of the carotenoid contents of chilli more than 80%. Gregory (1996) reported that oxidation caused a loss of ascorbic acid in chilli by using sun drying, because dried chilli was exposed directly to light and sun. Daood *et al.* (1996) reported that decrease ascorbic acid content of red chilli during sun drying was more destructive than the hot-air drying.

1.2.3.2 Hot air drying

Hot air drying has become more popular for drying chilli due to short drying time, uniform heating, and more hygienic characteristics (Chung *et al*., 1992). A temperature of hot air drying at 50-60^{o}C reduces drying time to less than 20 hrs. However, hot air drying is still accompanied by browning reactions and colour degradation in chilli (Mínguez-Mosquera *et al*., 1994). Krajayklang *et al*. (2000) reported that a dark-brown red colour chilli form when using the hot air drying at 60^{o}C for 6 hrs or 40^{o}C for 48 hrs. Berke and Shieh (2001) reported that the temperatures in hot air drying between 60 and 70ºC give maximum colour values and stability colour of dried chilli (with approximately 10% moisture content). Pordesimo *et al.* (2004) also reported that hot air drying at 65^{o}C was an effective drying jalapeño chilli to get a final moisture content equal to 6% with good stability of capsaicin content.

1.2.3.3 Freeze drying

Freeze drying is the best method of water removal with highest quality of a final product compared to other drying methods (Irzyniec *et al.,* 1995). Freeze drying is based on the dehydration by sublimation of a frozen product. Due to the absence of liquid water and low temperature required for the process, most of deterioration and microbiological reactions are stopped which give a final product of excellent quality. The solid state of water during freeze drying protects with minimal reduction of volume. Despite of many advantages, freeze drying has been recognized

as the most expensive process for manufacturing a dehydrated product (Ratti, 2001). Mahanom *et al.* (1999) reported that herbal preparation by using freeze drying gives superior phytochemicals content than oven drying at 50°C for 9 hrs. or 70°C for 5 hrs. Freeze drying was reported as the best method for retention of chilli colour. It is the most suitable drying method for maintaining the chilli colour quality (Park and Kim, 2007). However, the effect of drying methods on flavour compounds of chilli were less studied.

1.2.4 Dried chilli qualities

Dried chilli is widely used as a food ingredient for its hotness and colour. The most important qualities of the dried chilli are the colour and flavour (Kim *et al.*, 2002). Dried chilli has been mostly produced by red chilli. The red colour of chilli is mainly due to carotenoids. However, in many cases dried product becomes brown during drying and this reduces quality (Irzyniec *et al.,* 1995; Ratti, 2001). Since manufacturing procedure is affected on final moisture content, it is clear that the drying method will have an important influence on pigment stability (Ramesh *et al.,* 2001). Nonenzymatic browning reactions in foods are mainly affected on moisture content. The nonenzymatic browning reaction rate increases with higher temperatures and moisture content (Klieber, 2000).

The quality of dried chilli is assessed by a number of different parameters. Colour, hotness (capsaicin content) and ascorbic acid are the most obviously assessed parameters, but moisture content is also important due to its effect on pigment stability (Kanner *et al.,* 1977; Osuna-Garcia and Wall, 1998). In addition, some research is also focusing on the volatile flavour as important quality of dried chilli (Ruth *et al*., 2003; Jiang and Kubota, 2004; Yaldiza *et al*., 2010). Therefore, the main qualities discussed here are focused on colour, hotness, volatile flavour, moisture content and ascorbic acid.

1.2.4.1 Colour

Colour is the one of most important qualities of chilli, which affects the consumers' preferences. Undesirable changes in the colour may lead to a decrease in its quality and marketing value. Therefore, the surface colour of the chilli is an important criterion (Klieber, 2000). Chilli (*Capsicum annuum*) quality is

commercially evaluated with its strength of red colour. The intense red colour of chilli is controlled by carotenoids, mainly capsanthin, capsorubin and xanthophyllys (Irzyniec *et al.,* 1995; Ratti, 2001). Carotenoids are sensitive to light and temperature and are readily decomposed after trituration and extraction, respectively (Mínguez-Mosquera *et al*., 1994). The polyene structure renders carotenoids sensitive to heat, light and pro-oxidant conditions (Schiedt and Liaaen-Jensen, 1995). In particular at higher temperatures, it may occur undesired reactions of carotenoids. Vega-Gálvez *et al.* (2008), Turhan *et al*. (1997) and Lee *et al*. (1991) who suggested that using high temperatures (>70^{o}C) for hot air drying air results in a dark-brown colour due to non-enzymatic browning reaction. The colour loss relates to the formation of brown compounds during drying, since chilli contains considerable amounts of reducing sugars and amino acids, that could be active under high temperature (Lee *et al*., 1991; Turhan *et al*., 1997; Gógus and Eren, 1998).

1.2.4.2 Hotness

Hotness is the unique pungent flavour of chilli. The intense of chilli hotness depends on the concentration of capsaiciniond, particularly capsaicin (Wang *et al*., 2009). The stability of capsaicin depends on its intensity and duration of thermal treatment (Ornelas-Paz *et al*., 2010). Wang *et al.* (2009) reported that oven drying at 50°C for 8 hrs. and then heating at 100^{o}C for 15 min decreased the amounts of capsaicin in dried chilli (*Capsicum annuum*) less than 2%. Whereas, Pordesimo *et al.* (2004) reported that the drying temperatures ranged from 27 to 85^{o}C did not affect capsaicin content in dried jalapeño chilli (final moisture content 6%). Capsaicin content, in dried jalapeño chilli by using hot air drying at 65^{o}C until the final moisture reached 6% was increased about 15 times compared to fresh jalapeño chilli (Pordesimo *et al.*, 2004). This is due to the peroxidase enzyme by catalytic activity in fresh chilli which is inactivated that enzyme by blanching (Schweiggert *et al.*, 2006; Topuz *et al.* 2011)

1.2.4.3 Volatile flavour

Since volatile flavour is a sensory attributes, which was used instinctively for helping in the food selection. One factor responsible for the changes in volatile flavour compounds are the processing techniques, such as drying.

Kaminski *et al.* (1986) found the losses in volatiles in carrots approximately 69% and 75% with using hot air-drying, respectively. Moreover, Raghavan *et al*. (1995) reported that hot-air (50^{o}C) and freeze-drying did not affect volatile flavour compounds of thyme, whereas most compounds were lost under sun drying (in the shade). Less information is available on the effect of drying on the flavour volatile compounds in chilli. Luning *et al.* (1995) investigated the effect of hot-air (65^{o}C) drying on the volatile compounds in chilli (*Capsaicum annuum*.) and found that the compounds such as β-ocimene, hexanol, 3-hexenol and heptyl 6-methyl-2-propenoate decreased during drying, while compounds such as (Z)-2-pentenal, (E)-2-pentenal, (Z)-3-hexenal, (Z)-2-pentenol, (E)-2-pentenol, (E,E)-2,4-hexadienal, (E,Z)-2,4-hexadienal and 5-ethyl-2(5H)-furanone completely disappeared. However, some compounds formation after drying such as 2-methylpropanal, 2- and 3-methylbutanal, 2-methylfuran and 2,3-pentanodione were detected. The first three compounds come from the Strecker degradation of the α-amino acids valine, leucine and isoleucine, respectively. The other two are formed as intermediaries in Maillard reactions. Van Ruth (2001) and Van Ruth *et al.* (1995) identified Strecker aldehydes such as acetaldehyde, 2-methylpropanal, 2-methylbutanal and 3-methylbutanal in dried sweet chilli (*Capsicum annuum*.) and noted that these aldehydes could be used as an indicator for Maillard reaction. They also found dimethyl sulfide and 6-methyl-5-hepten-2-one in the dried sweet chilli.

1.2.4.4 Moisture content

Moisture content is a critical factor in the maintenance of pigment intensity (Lee *et al.,* 1992). The moisture may protect carotenoids from free radicals which are produced during pigment oxidation (Osuna-Garcia and Wall, 1998). Lee *et al.* (1991) reported that the 18% moisture content could lead to non-enzymatic browning and a potential risk of microbial growth. Lee *et al*. (1992) reported that moisture content between 10 and 14% retarded colour loss, while moisture content at lower than 8% accelerated pigment destruction. Wall and Bosland (1998) reported that final moisture content at 8% is ideal, as moisture content above 11% allows mould growth and below 4% causes excessive colour loss. Generally, dried chilli is needed moisture content less than 13% (Pitt and Hocking, 1997). This level also is the

regulatory moisture content of Thai dried chilli according to Thai Industrial Standards Institute (TISI 456, 1983).

1.2.4.5 Ascorbic acid

Chilli is a good source of ascorbic acid (Vitamin C). It helps to prevent oxidative stress-mediated chronic diseases such as cancer, cardiovascular disease, hypertension and neurodegenerative disorder. This is due to its antioxidant activity in the absence of toxicity (Lee *et al*., 2003). In addition, ascorbic acid helps to prevent pigment oxidation and enhances nutritional value (Biacs *et al*., 1992). Ascorbic acid has been mostly destructed for severe drying, and the quality of commercial red chilli products must be decreased in ascorbic acid contents (Kim *et al.*, 2006). Vega-Gálvez *et al*. (2008) reported that hot air-drying temperature had a detrimental effect on the retention of ascorbic acid. The inherent exposure of products to heated air induced oxidation, which caused the decrease of ascorbic acid content. Howard *et al.* (1994) reported that 75% of ascorbic acid in chilli was lost during drying, with the final content of ascorbic acid being in the range of 12.0-44.4 mg/100 g (dry basis). Ascorbic acid was oxidized by the high temperature during drying leading to L-dehydroascorbic acid (DHAA) and a wide variety of carbonyl and other unsaturated compounds being formed. The breakdown products then participated in Strecker degradation with amino acids and further polymerized to form melanoidins or non-nitrogenous caramel-like pigments (Gregory, 1996; BeMiller and Whistler, 1996).

1.2.5. Tools for analyzing pungent odour and hotness compounds of chilli products

1.2.5.1 Pungent odour compound analysis

Pungent odour compound is a volatile flavour of food that great importance in food acceptance and preference. All volatile flavour compounds are relatively small (< 400 Da) (Landy *et al.*, 1996). The volatile flavour compounds vary widely including, acids, neutral compounds, sulfur, nitrogen compounds, alcohols, aldehydes, ketones, hydrocarbons and esters. There are also large differences in the volatility of volatile flavour compounds, ranging from components with boiling points below room temperature to compounds that are solid at room temperature (Parliment, 1997). Small molecules such as ethanol, propanol, butanol, 1-penten-3-one,

acetaldehyde, propionaldehyde, acetic acid, propionic acid and butyric acid are highly volatile and exhibit pungent, harsh or chemical odour characteristics (Sun-Pan *et al*., 2007).

Many different analytical methods have been developed to determine fresh and processed chilli flavour, such as static headspace, dynamic headspace (Luning *et al*., 1994a, b), liquid-liquid extraction (LLE), simultaneous distillation extraction (SDE) (Chitwood *et al*., 1983; Korany *et al*., 2002) and solid phase microextraction (SPME) (Mazida *et al*., 2005; Junior *et al*., 2011). Static and dynamic headspace techniques have been extensively used (Piggott and Schaschke, 2001; Ruth, 2001). Static headspace technique is simpler, faster and solvent free. However it has low sensitivity and risk of cross-contamination (Mazza and Cottrell, 1999). The dynamic headspace technique is equilibrium between sample and headspace is constantly adjusted, resulting in improved sensitivity. The technique uses high temperatures which will yield extracts containing more components, but it may provide thermal decomposition (Guadayol *et al*., 1997). Using the SDE may change some compounds in a sample, which can be susceptible under steam distillation. LLE can result in the losses of volatiles with boiling points similar to the extraction solvent during removal of solvent by distillation (Mahattanatawee *et al*., 2005; Andujar-Ortiz *et al*., 2009). SPME shows some advantages over the other techniques. SPME is a rapid, simple, possible of working with small amounts of sample, adequate sensitivity, inexpensive, solvent-free technique, very suitable for analysis of the volatile and semi-volatile compounds in different types of sample. In addition, the use of this technique without the lengthy use of organic solvents or high temperatures in the extraction and concentration stages decreases the possibility of forming artifacts in the fraction extracted (Kataoka *et al*., 2000). However, SPME volatile concentrations are relatively low and thus difficult to analyze by MS when splitting the sample with a GC column (Mahattanatawee *et al*., 2005).

With the volatile components belong to many chemical families and show specific features, such as different polarity, solubility, volatility, stability, oxidation and degradation among others. Thus, more extraction techniques have been combined to be employed for ensuring full characterization of the volatile profile of a sample (Taveira *et al*., 2009; Domínguez and Agosin, 2010). The LLE and SPME

methods had been both recommended to extract the fruit for analysing volatile flavour profile by GC-MS (Mahattanatawee *et al*., 2005; Kuwayama *et al*., 2008; Domínguez and Agosin, 2010; Lee *et. al*., 2012). LLE is a traditional method which is still effective and remained in wide use (Raikos *et al*., 2009). It is a direct extraction method in which a liquid sample and an organic solvent are in contact. The LLE technique often involves a number of processes. First, the component mixture is dissolved in a suitable solvent and a second solvent that is immiscible with the first solvent is added. Next, the contents are thoroughly mixed (shaking) and the two immiscible solvents allowed separating into layers. The less dense solvent will be presented at upper layer, while more dense solvent will be in a lower layer. The components of the initial mixture will be distributed amongst the two immiscible solvents as determined by their partition coefficient. A compound that is more soluble in the less dense solvent will preferentially reside in the upper layer. Conversely, a compound more soluble in the more dense solvent will preferentially reside in the lower layer. Lastly, the two immiscible layers are separated, transferred and the component in that solvent is isolated by solvent evaporation and/or crystallization (Mohrig *et al*., 2010). The principle of LLE is based on the solubility of the volatile compound in the solvent, whose density should be different from that of water and immiscible in it (Wells, 2003; Solis-Solis *et al*., 2007). Importantly, the solvents are selected based on its selectivity and boiling point, and must be of high purity (Sugisawa, 1981). When extracting volatiles directly from sample, polarity of the solvent must match that of the target volatile compound (Maarse and Belz, 1981). Most extractions involve water because it is highly polar and immiscible with most organic solvents. In addition, the target compounds must be soluble in the organic solvent, but insoluble in the water layer (Wells, 2003). Organic solvents frequently employed for LLE procedures, namely diethyl ether, hexane, benzene, dichloromethane and pentane (Mohrig *et al*., 2010). Whereas, some researchers propose LLE with a mix of dichloromethane and pentane (1:2 v/v) as extraction agent for fruit samples (Ibarz *et al*., 2006; Oliveira *et al*., 2008; Genisheva and Oliveira, 2009). Diethyl ether (ether) is an effective extraction solvent and with its low boiling point (37°C) is widely used, however, the high volatility and extreme flammability of diethyl (Mohrig *et al*., 2010). Hexane is suitable for extraction of non-polar

compounds such as aliphatic hydrocarbons (Darmawan *et al.,* 2012). Benzene is suitable for aromatic compounds (Smallwood, 1996). Dichloromethane has high extraction efficiency for a wide range of non-polar to polar compounds (Laohaprasit *et al*., 2011; Popescu *et al*., 2011). Its boiling point is low (39.75°C) and easy to reconcentrate after extraction (Smallwood, 1996). It is easy to separate from water because of its higher specific gravity, and it is non-flammable (Mohrig *et al*., 2010). Whereas, pentane is a non-polar solvent commonly effective at extracting non-polar compound at low levels such as ethanol, and it also has an affinity for esters and fatty acids (Smallwood, 1996; Martín-del-Campo *et al*., 2011).

SPME is a technique based on physicochemical processes of equilibrium between the matrix and headspace and between headspace and the material coating the fibre (Nongonierma *et al*., 2006). Efficiency of SPME depends on selection of fibre coating. Several types of coating fibres are currently available for the extraction of aroma. The affinity of the fibre for an analyte depends on the principle of 'like dissolves like'. Coating fibres with non-polar polydimethylsiloxane (PDMS) is preferred to extract non-polar analytes such as volatile flavour compounds. However, it can be applied successfully to more-polar compounds, particularly after optimizing extraction conditions. PDMS is very rugged and is able to withstand at high injector temperatures, up to about 300^{o}C. The polar polyacrylate (PA) fibre is preferred to extract more-polar analytes. Mixed coating fibres, containing divinylbenzene (DVB) copolymers, templated resin (TPR) or Carboxen (CAR: a porous activated carbon support), increase retention capacity due to the mutually potentiating effect of adsorption and distribution to the stationary phase (Kataoka *et al*., 2000). PDMS/DVB, CAR/DVB and CW/TPR can be used for the extraction of volatile low molecular mass and polar analytes (Mani, 1999). CAR/PDMS fibre shows high capacity for concentrating volatile components from the headspace of foods, which were heated to high temperature (Brunton *et al*., 2001). DVB/CAR/PDMS fibre provided the great number of volatile compounds of chilli. The intermediate polarity of this fibre is probably related to its capacity to extract a great number of volatiles. Since the rugosity of the PDMS film results existence of meso-macropores which associated with the solid pores of CAR and DVB (Junior *et al*., 2011). The SPME coupled with GC-MS is indicated as an excellent method for

analysis of the volatile flavours of spices. However, optimization of the time and adsorption temperature is required to achieve good performance in the extraction and results of high reproducibility. High temperature can be used to improve the extraction efficiency of volatile compounds (Zhang *et al*., 1994). Mazida *et al*. (2005) reported that extraction at 60^{o}C for 30 min by SPME (DVB/CAR/PDMS coated fibre) analysis gave the highest yields of volatile flavour substances from chilli (*Capsicum annuum*). While, Junior *et al*. (2011) reported that the highest number of volatile flavour compounds of chilli and peak area were obtained at a temperature 40^{o}C with an extraction time of 80 min by SPME (DVB/CAR/PDMS coated fibre). These differences make different volatile flavour patterns between LLE and SPME methods, and the combined methods can improve reliability of the profiling results (Lee *et al*., 2012). Therefore, the LLE and SPME methods are both used to assure that the results of GC-MS were representative of volatile flavour compound of chilli.

1.2.5.2 Hotness compound analysis

Hotness is defined as a total intensity and duration of burn sensation in the throat and in the mouth (tongue, palate and chick mucosa) perceived during and after ingestion (Reinbach *et al.*, 2007). Chilli hotness has been attributed to a family of compounds called capsaicinoids, particularly capsaicin (69%) (Kuraian and Starks, 2002). Reversed phased High Performance Liquid Chromatography (HPLC) analysis is the most widely used by many researchers for capsaicin analysis. It is an accurate method of calculating hotness caused by capsaicin content in a food product (Sanatombi and Sharma, 2008; Cooper *et al*., 1991). Cooper *et al*. (1991) developed a reversed-phase C-18 column of HPLC to separate each capsaicinoid present in chilli (capsaicin and dihydrocapsaicin), and used UV detector at 280 nm with the mobile phase of methanol/water (60:40 v/v) with a flow rate of 1.5 ml/min. It was found that the retention times of capsaicin and dihydrocapsaicin were 16.8 min and 28.0 min, respectively. Betts (1999) determined capsaiciniods namely, capsaicin and dyhidrocapsaicin in hot chilli sauce samples by using a C-18 column, and UV detection at 284 nm with the mobile phase of methanol/water (80:20 v/v) with a flow rate of 1.5 ml/min and found that the retention times for capsaicin and dihydrocapsaicin were 3.19 and 3.96 min, respectively.

Generally, chilli hotness is expressed in SHU (Scoville, 1912). The hotness (or heat factor) of chilli is measured in multiples of 100 units. Chilli hotness ranges from 0 SHU for a bell chilli to 300,000 SHU for a habanero chilli, according to the American Society of Testing and Materials (ASTM). One part of chilli hotness per 1,000,000 drops of water is rated at only 1.6 SHU. The substance that makes a chilli hot is called capsaicin. Pure capsaicin rates between 15,000,000 and 16,000,000 SHU. The chilli's hotness in SHU was calculated by using SHU equation, its equal to amount of capsaicin (% dry weight) multiple by 160,000 (Govindarajan, 1986). Table 3 is a list of different chilli types that reported the hotness in terms of relative heat levels and their SHU.

Table 3 A relative hotness scale of different chilli types

Name	Species	SHU
Orange Habanero	chinense	210,000
Red Habanero	chinense	150,000
Tabasco	annuum	120,000
Chiltepin	annuum	75,000
Thai hot	annuum	60,000
Serrano	annuum	25,000
Long Slim Cayenne	annuum	23,000
Mitla	annuum	22,000
Santa Fe Grade	annuum	21,000
Aji Escabeche	bacatum	17,000
Long Thick Cayenne	annuum	8,500
Jalapeño M	annuum	5,500
Numax Primavera	annuum	5,000
Numax Sandia	annuum	5,000
Numax Joe E. Parker	annuum	4,500
Pasilla	annuum	4,000
Mutalo	annuum	1,000
Bell	annuum	0

Source: New Mexico State University (2006)

The hotness level in chilli was classified into 3 groups, according to Scoville Heat Unit (SHU) (Tepsomboon, 1997; Govindarajan, 1986).

1. Very hot chilli, this chilli has heat rate between 70,000-16,000,000 SHU. The chilli has small pod and uses for volatile oil extraction such as Tabasco.

2. Medium hot chilli, this chilli has heat rate between 35,000-70,000 SHU. This chilli is used as an ingredient or added in spicy foods such as Chee fah (*Capsicum annuum*.) and Chinda (*Capsicum annuum*.) that belong to Thai's chilli.

3. Less hot or non-hot chilli, this chilli has heat rate between 0-35,000 SHU. This chilli has a large pod, pear-or egg-shaped pods, such as sweet and bell chillies.

Another study was done by Weiss (2002) who classified the hotness level into 5 categories as following.

1. Non-hot is heat rate between 0-700 SHU.
2. Mildly hot is heat rate between 700-3,000 SHU.
3. Moderately hot is heat rate between 3,000-25,000 SHU.
4. Highly hot is heat rate between 25,000-70,000 SHU.
5. Very highly hot is heat rate more than 80,000 SHU.

1.2.5.3 Sensory evaluation

The traditional method of determining hotness of chilli and food preparations is tasting. (Cázares- Sánchez *et al.*, 2005). Scoville (1912) developed the method to measure the heat level of chilli called Scoville Organoleptic Test, to measure the hotness of chilli. A panel tasted a chilli sample and then recorded the heat level. The sample was diluted until its hotness could not be increasingly detected. In the original test, Scoville would take an extract from a chilli and determine how much sucrose solution was required to dilute it before its hotness could no longer be detected by a panel. For example, if he had 1 g of ground chilli, and it took 100 ml of sucrose solution to dilute it until its hotness was no longer detectable, then it would rank at 100 SHU. If it took 1000 ml of sugar water to dilute 1 g of of ground chilli, then it would rate 1,000 SHU (Rohrig, 2014).

The simple scale of 0-10 is often used to evaluate heat scale of chilli types, with 0 and 10 being mildest and the hottest. The degree of hotness (heat or bite) is determined by the amount of capsiacinoids (Bosland, 1996) (Table 4).

Table 4 A relative hotness score level of chilli types from the mildest to the hottest

Score level	Chilli types
0	Sweet Bell, Nemex Conquistador Chile, Paprika
1	Anahieim Mild
2	NuMex R Naky
3	New Mexico 6-4
4	NuMex Big Jim
5	Sanda
6	Barker's Hot, E.spañola Improved
7	Jalapeño
8	Cayenne, Tabasco
9	Chie piquin
10	Habanero

Source: Bosland (1996)

Note: 0 is the meaning of the mildest score and 10 is the meaning of the hottest score.

1.2.5.3.1 Sensory panellists

In sensory evaluation, one of many questions regarding to the panellist is how many people are needed for sensory panel. Sheehy (2009) mentioned that there is no ideal number of panellists and it is up to the researcher to consider each case separately, taking into account type of the testing as well as the samples. Having a larger number of panellists increases statistical reliability by reducing the effect of between-person variability. But it can cause difficulties for the researcher in terms of workload as well as making it more difficult to communicate. On the other hand, having too few panellists can cause the study open to influence unduly of judgements by one panellist, who is out of step with the other panellists. Lyon *et al*. (1992) claimed that at least five panellists should always be used for a given test and that if the test is replicated a few times this number can give satisfactory precision for most situations. However, a larger number of panellists (e.g. 10-20 panellists) seem preferable to use to cut down on the amount of replication. One point of worth stressing is a selected panellist must be properly qualified to achieve a determination (Sheehy, 2009).

Considering panellist characteristics in sensory evaluation, panellist qualifications vary depending on the test. All panellists participating in analytical tests are generally expected to be more sensitive to differences in tested products than normal consumers. Therefore, a set of screening exercises should be administered for panellist selection (Meilgaard *et al.,* 1999). Often, these exercises include discrimination tests where the rate of correct answers for satisfactory panellists should be well above the chance probability for tests employed (e.g. p=1/3 for triangle tests or p=1/2 for duo-trio tests) (Meilgaard *et al*., 1999; Lawless and Heymaan, 2010). The criteria to evaluate panellists sensory sensitivity varies depending on the difficulty of the screening test or the requirements of the project (Stone and Sidel, 2004). The panellists of descriptive test are expected to have excellent verbal abilities, which are important for the consensus language development during training sessions (Meilgaard *et al*., 1999). The mission of affective tests is to differentiate the products based on consumer likings or preference levels. The panellist in preference tests must be actual product users and likers, and able to express their acceptance levels for products differently in each category (Meilgaard *et al*., 1999; Stone and Sidel, 2004).

The characteristic of panellist in sensory evaluation has been considered base on demographic model. The demographic criteria are described to follow in many justifications for the later stages of sensory method (Stone and Sidel, 2004). Several demographic criteria have been used to screen panellists, as following.

- **Age**: Decreased taste perception with age has been reported (Schiffman, 1997; Fukunaga *et al*., 2005; Kennedy *et al*., 2010). Decreasing taste sensitivity with age was once thought to result from a decrease in the number of taste buds (Fukunaga *et al*., 2005). Mojet *et al*. (2001) reported that the elderly (60-75 years) panellists show a large variability of thresholds. Sugimoto (1994) reported that taste receptor cells undergo continual turnover, with a life span of approximately 10 days in young animals, which may serve to maintain taste sensitivities. Therefore, one possible mechanism of age decline is the time lag of this turnover, resulting in deterioration of taste cell responses. Decrease odour perception with aging has been reported. Elderly panellists exhibit a lower sensitivity for odours intensity, as reflected in absolute threshold measurements (Cain and Gent, 1991) and in intensity measures of suprathreshold odours (Stevens and Cain, 1985). Shusterman *et al*. (2003) reported

that the younger age (18-34 years) panellists had higher sensitivity of volatile organic compound (VOC) and CO_2 samples than the middle (35-51 years) and older age (52-68 years), respectively. Larsson and Bäckman (1993) reported that elderly panellists (60-69 years) had poorer recognition memory in odours than young panellists (19-34 years). Cain and Gent (1991) and Stevens and Cain (1985) reported that the sense of odour is more impaired by aging than the sense of taste. Schiffman1and Graham (2000) suggested that the olfactory cells undergo continual turnover, with a life span of approximately 30 days. The odour perceptual loss during aging are due to changes of anatomic and physiological in the olfactory epithelium (the olfactory bulb and nerves) and higher levels of the brain (including hippocampus and amygdaloid complex and hypothalamus) that receive olfactory input.

- **Gender**: Some researchers reported that gender has a difference in odour perception (Mojet *et al*., 2003; Ohla and Lundström, 2013). Females have greater sensitivity and/or greater physiologic responsiveness to stimuli in a number of sensory modalities (Bartoshuk *et al*., 1994; Ohla and Lundström, 2013). Dalton *et al*., (2002) stated that females exhibited greater gustatory and olfactory sensitivity than males. It is still unknown why females show this superiority, but gender-related differences in factors, such as hormones (estrogens, progesterone), environmental background and verbal fluency might play a role (Ship *et al*., 1996). It has been less investigated the effect of gender on chemesthetic sense. Cometto-Muñiz and Noriega (1985) found that females showed more sensitive than males, at least when the stimulus is presented nasally. Whereas, Olofsson and Nordin (2004) investigated chemosensory gender differences by rating means of unpleasantness and nasal irritated perception for three concentrations of pyridine. The 19 females and 17 males of panellists were recruited. It was found that a tendency of higher unpleasantness ratings in females than in males. However, there was no main effect of gender in sensory irritated perception ($P>0.05$).

- **Personal characteristics**: The personal characteristics are classified as personal ability, personal experience with food and personal liking on food. The details will be discussed.

- **Personal ability:** There is natural variation in the ability of people to smell/taste – some have no sense, some are medium sense, and some are extremely

sensitive to smell/taste (Bartoshuk, 2000; Drewnowski, 1991; Drewnowski *et al*., 2001). Bartoshuk *et al*. (1995), found that people have genetically developed into classes of tasters, whereby their taste receptors are different. There are three groups of tasters: supertasters who endure the most intense taste sensations, medium tasters who perceive intermediate taste intensities and non-tasters, who perceive the weakest taste intensities (Bartoshuk *et al*., 2004). Bartoshuk *et al*. (1994) noted that the tongue anatomy of tasters is different from non-tasters, with supertasters tending to have the most taste pores and fungiform papillae. Once more, the taster status is correlated with self-reported taste magnitudes and food preferences. For example, it has been found that the perception of sucrose is sweeter for tasters than non-tasters (Bartoshuk *et al*., 2004). Concerning the perception of bitter foods and beverages, the tasters of PROP showed more disliking to foods such as grapefruit juice, green tea and some beers than the non-tasters (Akella *et al*., 1997). Supertasters perceive PROP with more intense than the non-tasters. Supertasters also perceive tastants, such as NaCl with more intense than medium-tasters and non-tasters, respectively (Bartoshuk *et al*., 1998). They also perceive the sourness of citric acid to be more intense than the medium-tasters and non-tasters (Prescott *et al*., 2001). Furthermore, supertasters are more likely to have intense feelings of oral pain and irritation. Because capsaicin affects heat receptors (VR1) and thus may suppress other unpleasant tastes. Pain caused by chilli can be quite pleasant, because damage in tissue is not involved (Szallasi and Blumberg, 1999). Differences among the taster groups have been observed for trigeminal sensations, including irritation from alcohol, capsaicin and the burn of chilli (Karrer and Bartoshuk, 1991; Bartoshuk *et al.*, 1994; Lucchina *et al*., 1998).

- **Personal exposure with food:** Exposure with food may also influence on sensory perception. Many studies have demonstrated a reduction in the perception of a given stimulus over time. The gradual reduction of sensation or neuronal/receptor activity during sustained stimulation of constant intensity is usually defined as adaptation (Price, 1992; Theunissen *et al*., 2000). Perceptual adaptation has been observed in virtually all modalities including taste (Theunissen *et al*., 2000), smell (Dalton, 2000) and heat pain (Price *et al*., 1977). The people who had recent experiences are induced to sense adaptation greater than the people who had

temporally distant experiences (Helson, 1964). The repeated exposure to high chilli content in dish over 2 weeks caused decremented response (Stevenson and Prescott, 1994; Prescott and Stevenson, 1995b).

The difference in an individual consumer in frequency of consumption spicy food had an effect on the intensity evaluation of oral burn attribute (Cowart, 1987; Lawless *et al*., 1985; Lawless *et al*., 2000; Stevenson and Prescott, 1994; Prescott and Stevenson, 1995b). Consumer can be classified into two groups of chilli users namely, non-chilli user is who ate spicy foods less than once a month and chilli-user is who ate spicy foods at least three times a week. (Lawless *et al*., 2000; Prescott and Stevenson, 1995b; Reinbach *et al*., 2007; Ludy and Mattes 2012). Whereas, some researchers divided consumers into the light, moderate and heavy users (Hoefkens and Verbeke, 2013; Reckitt Benckiser plc, 2013). Heavy users used spicy food (e.g. hot or Tabasco sauce) every day, moderate users used the spicy food once a week and light users can be defined as using spicy food less often (Reckitt Benckiser plc, 2013). Stevenson and Prescott (1994) mentioned that people who regularly consume spicy foods are partially desensitized to the sensory effects of oral burn. They are much less responsive above threshold. Prescott and Stevenson (1995b) stated that chronic desensitization by capsaicin may produce chronic decrements in taste or odour intensity. Capsaicin is a neurotoxic and regular consumption may cause damage, especially the unmyelinated nerve receptors and the leading fibres to chronic desensitization (Duner-Engstrom *et al.,* 1986).

- Personal liking on food: Hedonic and intensity ratings were also combined with the frequency of consumption for classifying the consumers in some researches (Lawless *et al*., 1985; Stevenson and Yeomans 1993; Lawless *et al*., 2000; Ludy and Mattes, 2012). The regular chilli-users have more favourite in spicy food and less rating on oral burn intensity, when compared with non-chilli users (Lawless *et al*., 2000; Ludy and Mattes, 2012). Wardle *et al*. (2003) stated that the familiarity or experience or exposure may lead to enhance food likes. The more frequently a food is tasted, the more it is liked. An exposure to a target food once a day for ten days can dramatically increase intake of the target food and intake may nearly double after only one exposure (Wardle *et al*., 2003).

1.2.5.3.2 Descriptive analysis

Descriptive analysis technique involves the characterization of the product by attributes and the intensity of those attributes. It has qualitative and quantitative components. Both are necessary for the effective performance of the data analysis (Muñoz and Civille, 1998). A generic descriptive analysis would usually used trained panellists. These panellists would not be asked for their hedonic responses to the products (Lawless and Heymann, 2010). An important procedure of descriptive sensory analysis is lexicon development. This procedure is used for product development, quality control and in laboratory practices (Civille and Lawless, 1986). The important thing which is considered for lexicon development is to have a consensus among the panellists in all terms and definitions.

Descriptive analysis can integrate product attributes as far as consumers' descriptive language is concerned. This means that descriptive analysis is consisted of training a panel (approximately 6 to 12 panellists) to identify and quantify specific sensory attributes in order to help or match consumer tests (Drake *et al*., 2007). Having a set of reference standards is necessary for stabilizing the panellist's use of vocabulary. It is also useful for assisting in the training. The reference standards are helpful to decrease judge variability, to counterbalance cultural differences in interlaboratory studies and to allow calibration of the panellist in using intensity scale (Stampanoni, 1993a, b; Lawless and Klein, 1991). Generally, the reference standards are chemicals, spices, extracts ingredients and finished products, which are used to specify a selected characteristic of a product (Stampanoni, 1993a, b). Reference standards should be simple, reproducible, diluted without changing character, very clear to the subjects and very specific (Rainey, 1986). Not all attributes are so easily described by an ideal reference standard. Sometimes, a single standard is not enough for proper concept alignment (Stampanoni, 1994).

Descriptive analysis technique has been used to focus on the description and quantification of the flavour of spicy products. Allison *et al*. (1999) used descriptive analysis to evaluate hotness of tomato-based salsa (contained 7.3 ppm capsaicin) with varying interstimulus intervals, with and without rinsing. Seven samples were tested daily with variations in intervals between stimuli (30 s, 1, 2, 4, 8, and 16 min). Five panellists were trained more than 120 hrs. The panellists were given

7 g samples and tested by using both systems of no rinse and rinse their mouths. In no-rinse regimens, the first sample was taken at 0 s, chewed and swallowed by 10 s**,** and then rated 15 s following initial ingestion. In rinse regimens, all procedures were identical, except that the panellists ate a cracker and sipped water between each rating for each sample. They were instructed to evaluate the samples on a 15 cm line scale. It was found that rinsing significantly increased repeatability and increased the rate of heat decay ($P \leq 0.05$). Exponential heat decay was observed. Tongue heat was significantly higher than oral cavity and throat bum. This study showed that seven samples of medium-heat salsa could be tested daily with at least 16 min between samples and liberally rinsing with crackers and water.

Cliff and Heymann (1992) used descriptive analysis to characterize irritation of the principal irritants of red chilli (capsaicin), black pepper (piperine), cinnamon (cinnumaldehyde), cumin (cuminaldehyde), cloves (eugenol), ginger (ginger oleoresin), and alcohol (ethanol). Twenty-one panellists participated in this study. The panellists participated in one of two round table discussion sessions for orientation and term development. At these sessions, panellists were introduced to the pungent solutions and a list of tentative terms. They were asked to describe the perceived sensations in terms of the quality, intensity, time and location in the mouth. All solutions were presented in dark-blue opaque glasses for masking an interfering of colour and odour. The panellists wore nose-clips during the evaluations for blocking a retronasal transferring of odour, thereby allowing the panellists to focus on the perceived mouth qualities. The panellist was given three samples in each session and asked to hold the entire sample (10 ml) in their mouths for 5 s, expectorated, waited 30 s and then evaluated the attributes. They were required to rinse with water and wait 5 min between samples. Attributes were scored on 15 cm unstructured line scales from none to extreme, whereas the temporal attributes (lag and duration) were rated from short to long. It was found that irritation of cinnumaldehyde was primarily burning and tingling and numbing. It had a quick onset and rapid decay. The irritation of eugenol had a long-lasting, predominantly numbing effect. The irritation of piperine, capsaicin and ginger were primarily burning, but had different temporal and spatial responses. The irritation of ethanol was most diffuse in nature, with some

burning and tingling sensations. It had the shortest perceived onset and overall duration.

Descriptive analysis technique has been used to combine with instrumental analysis for description and quantification of the flavour of spicy products. Mamatha *et al.* (2008) assessed odour and flavour quality of different cultivars of pepper (Panniyur 1, Balankotta, Panniyur 5 and one commercial sample) by descriptive and instrumental analysis. A group of 10-12 panellists were trained over three sessions for psychometric studies and Quantitative Descriptive Analysis (QDA) test. The panellists were trained to sniff the headspace and distinguish various odour notes. They were asked to list the flavour descriptors perceived by sniffing. The panellists were asked to mark the intensity of the perceived sensation on a 15 cm line scale. It was found that the flavour profile of the essential oils of pepper samples showed a higher intensity of pepper-like note in Panniyur 1, Panniyur 5 and commercial sample, and turmeric-like and green mango-like characterized Balankotta. The odour profile of the essential oils further supported the flavour profile data. GC-MS analysis complemented the sensory odour and flavour profiling results. The GC-MS of Balankotta pepper samples was different from Panniyur 1, Panniyur 5 and the commercial sample, showing higher content of *p*-cymene.

Another combining descriptive analysis and instrumental evaluations, Wortel *et al.* (2005) used both methods identified the characteristics of cosmetic products. Key characteristics of the cosmetic products were related to rheological properties using multivariate methods of analyses. The researchers demonstrated that the multivariate method clearly shows the relationship between rheology and sensory properties using the Partial Least Squares model (PLS), which is a regression method similar to PCA. Instead of maximizing the explained variance in the data set, PLS maximizes the explanation of the dependent value (*y*-value).

There are several different methods of descriptive analysis, including the Flavour Profile (Cairncross and Sjöstrom, 1950; Wortel *et al.* 2005), Texture Profile (Brandt *et al*., 1963), Quantitative Descriptive Analysis™ (Stone *et al*., 1974), the Spectrum™ method (Meilgaard *et al*., 1999), Quantitative Flavour Profiling (Stampanoni, 1993a, b), Free-Choice Profiling (Langron, 1983; Thompson and MacFie, 1983) and Generic Descriptive Analysis (GDA) methods. The

specific methods reflect various sensory philosophies and approaches (Lawless and Heymann, 2010). However, generic descriptive analysis, which can combine different approaches from all these methods, is frequently employed during practical applications in order to meet specific project objectives. The method provides the most detailed or complete description of products and/or product categories (Murray *et al.*, 2001).

- **Generic Descriptive Analysis**: It generally takes pieces from QDA and Spectrum™ methods, but is modified to suit the goals of the project and limitations of the product being tested (Lawless and Heymann, 2010). The GDA method would usually have between 8 and 12 trained panellists, with the use of reference standards, to understand and agree on the meaning of the attributes used. They would usually use a quantitative scale for intensity which allows the data to be statistically analyzed. GDA can be completed in three steps. These steps are composed of panellist training, panellist determining in reproducibility/consistency and sample evaluating of the panellist. GDA has three methods of the panellist training, namely consensus, ballot and a combination of consensus and ballot trainings. However, a combination of consensus and ballot training, which the panellists derive some descriptors on their own through consensus and others are added through suggestions by the panel leader or from word lists, is frequently used. During the final training session, the panellists create the score sheet and they may be allowed to decide on the scale to use.

Initially, the panellists are exposed to the entire range of the products. They are asked to evaluate the sensory differences among the samples and to write down the descriptors that describe these differences. This occurs in silence. When all panellists complete this portion of the assignment, the sequence of training session would be started (Lawless and Heymann, 2010).

In consensus training, the panel leader asks each panellist to list the words used to describe each sample. During this phase of the training, it is extremely important that the panel leader must be cautious not to lead or to judge any descriptor from any panellist. However, the panel leader may ask for clarification, if needed. Usually, the panellists themselves will begin to move toward initial consensus when they see the total list of descriptors elicited. Subsequently, the panel leader should attempt to provide potential reference standards based on the initial consensus. These

reference standards are chemicals, spices, ingredients, or products that can be used to help the panellists identify and remember the sensory attribute found in the samples evaluated (Rainey, 1986). In general, the panel leader should strive to use actual physical objects as the reference standards but in some cases precise written description may be used instead. Next session, the panellists are exposed to the samples again and asked to decide on the possible reference standards. If reference standards are not feasible, the panellists can also be asked to verbally define the specific descriptor. This refinement of the consensus list of descriptors, reference standards, and definitions continues until the panellists are satisfied that they have the best possible list and that everyone understands each term completely. During the final training session the panellists create the score sheet. They allow to decide on the scale to use. The panellists are asked to decide on the words needed to anchor the scales such as none to extreme or slight to very strong. Then, the panel leader will start to evaluate judge reproducibility

In ballot training, the panel leader gives each panellist a word list (or sample score sheet) for the products. The word list contains words, definitions and often the panel leader will also have reference standards available to anchor the descriptors. There are a number of published word lists (lexicons) available for a variety of foods and personal care products. A non-exhaustive list is given at the end of this section. The panellists are then asked to indicate through consensus which of these words, reference standards and definitions should be used in the specific study. The panellists are allowed to add or delete terms through consensus. They are also asked to sequence the descriptors on the ballot. In subsequent sessions the panellists are exposed to the samples again and asked to look at the ballot that they previously created. They then have to decide if this is truly the score sheet they want to use with these products. Refinement of the score sheet, reference standards, and definitions continues until the panellists are satisfied that, this is the best possible score sheet, best sequence, and that everyone understands each term completely. Now the panellist leader is ready to determine judge reproducibility (Meilgarrd *et al*., 1999; Lawless and Heymann, 2010).

Once the training section has been completed, the panellist performance is checked in reproducibility/consistency. In the final step, the panellists evaluate the samples at least 2-3 replications (Reilly and York, 2001).

- **Scaling:** There are a variety of sensory tools that can be used to assess the perceived intensity and acceptability of food products. Using scales with high discriminative power, good reliability and some predictive value for correlating with food habits is a goal of sensory evaluation. It is certainly possible that different scaling methods might induce different cognitive strategies or decision rules that vary in their discrimination efficiency (Lawless *et al*., 2010).

According to Meilgaard *et al.* (1999), scales are more informative compared with difference tests therefore a more useful is a form of recording the intensity of perception. Although the properties of data obtained from any response scale may vary with the circumstances of the test (e.g., familiarity of panellists with the attribute to be tested), it is typically assumed that:

(1) Category scaling yields ordinal (or interval) data.

(2) Line scaling yields interval data.

(3) Magnitude estimation (ME) scaling (often called ratio scaling) sometimes, but not always, yields ratio data.

Category and line scales have been used historically to quantify sensory or hedonic experiences (Bartoshuk *et al.,* 2003; Lim *et al*., 2009). Category scales partition intensity into bins that frequently have a numeric and/or semantic label (e.g., 1 = very weak, 5 = medium, 9 = very strong). Whereas line scale has no subdivisions. Rather, a line scale is typically an unstructured line scale anchored at its ends with the minimum and maximum ratings for a particular attribute (e.g., ‘not hotness’ to extremely hotness). These scales are purportedly straightforward and easily understood by panellists (Hayes *et al*., 2013). Unlike magnitude estimation, which asks panellists to express intensities in terms of ratios, requiring both training and a certain level of numeracy (Lawless *et al*., 2010). Line and category scales are also faster to use, and easier to understand than magnitude estimation (Hayes *et al*., 2013). A good discussion of the advantages and disadvantages of the ME and category scaling is given by Pangborn (1984). The data produced by ME have ratio properties, like the standard forms of technical measurement (length, weight, volume,

etc.). ME gets around the problem that panellists avoid the ends of scales to leave room for another stimulus. Supporters of ME also cite the fact that users of category scaling must spend time and effort on preparation of standards and on teaching the panellists to use them. Those favouring category scales note that ME is incapable of providing stable and reproducible values for flavour intensity.

Labelled Magnitude Scale (LMS): Because of differences between those scales, Green *et al.* (1993) empirically constructed a semantic scale of oral sensations that was called Labelled Magnitude Scale (LMS). This scale provides a good power of discrimination of data. It is not necessarily the hard training of panellists (Green *et al.,* 1993). The LMS might be considered to be more powerful to investigate flavour measurement intensity and can semantically understand intensity stimuli, as well as easy understanding for a panellist to use the scale (Cortez-Pereira *et al*., 2009).

LMS is a specialized line scale with semantic labels at empirically derived intervals which were rapidly adopted in chemosensory research. Unlike line scales and categorical scales, it generates data similar to ME (Green *et al*., 1993; Green *et al*., 1996) and is easier to use. LMS would have ratio properties and could be used to quantify all forms and intensities of oral perception. Their strategy was to obtain magnitude estimates of adjectives within the context of numerous recalled real life experience with stimuli from five different sensory modalities: taste, touch, temperature, smell and pain. The geometric means of the resulting estimates were then used to construct semantically LMS of oral sensation. The results were the means of the logarithms of the magnitude estimates given to the six descriptors and their associated 95% confidence intervals. The verbal descriptors, which are placed on the scale according to their associated geometric means (i.e., the antilog of the log means), are not evenly spaced. In the length 0 to 100, the position of the verbal labels on the LMS, as percentages of full scale length, are: barely detectable, 1.4; weak, 6.1; moderate, 17.2; strong, 35.4; very strong, 53.3; strongest imaginable, 100 (Green *et al.* 1996). The LMS constructed from these values is shown in Figure 4.

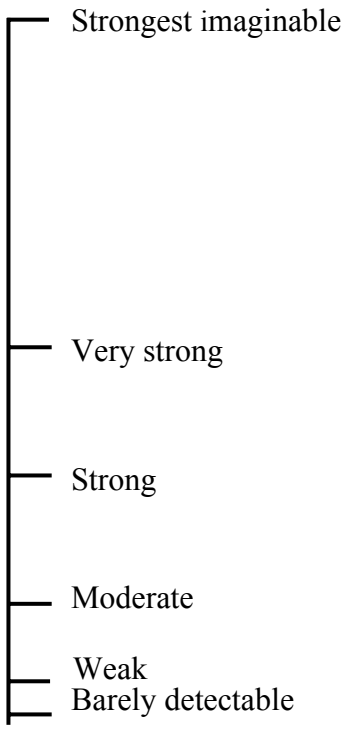


Figure 4 Labelled Magnitude Scale, with verbal anchors spaced to represent perceptual intervals as determined by ratio scaling instructions

Source: Green *et al.* (1993)

In setting up LMS scale, panellists provide magnitude estimates of different verbal descriptors after giving magnitude estimates of familiar oral sensation (Green *et al.,* 1993; Lawless and Heymann, 2010). The goal of LMS scale development was to provide an applicable tool of diverse sensory attributes, for example the sensations from oral irritants and sensation of taste (Lawless and Heymann, 2010). The key features of the LMS are the unequal, quasi-logarithmic spacing of its verbal labels and the presence of strongest imaginable at its upper bound (Green *et al.,* 1993). The use of these phrases was assumed to serve as a fixed end-point of sensation that aligns judgments of different panellists to a common sensory ruler and was used to avoid the practical issue of how to extremely accommodate large magnitudes on a line of fixed length (Cardello *et al.,* 2005). Panellists can quickly look at the verbal labels and corresponding numbers and place

a slash mark (/) through the line to indicate the perceived strength of their sensation with only minimal instruction.

Before the data to be analysed, the LMS scale is skewed in manner approximating a log-normal distribution and subsequent operations on the data sets were, therefore, performed on the logarithms of the means. The data are analysed using the repeated-measures ANOVA. The significance between the samples was evaluated with the comparison tests of mean.

In comparing with other scale, the LMS produced psychophysics effects identical to ME for measuring the intensity of odours or tastes (Green *et al.* 1993; Green *et al.,* 1996). Moreover, the LMS produced steeper functions than ME when panellists had been explicitly instructed to omit painful sensations (e.g., the burn of chilli) form the concept of strongest imaginable odour (Doty *et al.,* 1978; Kobal and Hummel, 1988). This could be concluded that the LMS can be used to scale sensations of taste and smell. The LMS was compared to category-ratio scale, developed to measure the physical strength. Cardello *et al*. (2005) demonstrated the sensitivity and reliability of two scales between LMS and category scales, which were used to rate levels of satiety (hunger/fullness) by panellists. It was found that LMS scale has greater sensitivity than category scales and an average reliability coefficient of 0.90. This indicated that LMS scale is a sensitive, reliable and easy-to-use scale for measuring perceived satiety that has several advantages over other, more commonly used satiety scale.

The LMS has repeatedly been proven to be a valid instrument to classify individual as tasters or non-tasters (Lucchina *et al*., 1998)**.** The top of the scale is labelled strongest imaginable. The mean rating of supra-threshold taste intensity was labelled strong. The LMS rests on the assumption that strongest imaginable refers to the same perceived intensity on average, across non-tasters, medium tasters and supertasters (Bartoshuk *et al*., 1998). Tasters are considered to report the supra-threshold concentrations of PROP as strong, extremely strong or strongest imaginable. Non-tasters rate PROP as barely detectable or weak (Reed *et al*., 1999). Whenever the detection of small perceptual differences among individuals is of interest, scales with ceiling effects should not be used. The LMS minimizes ceiling effects and therefore is better in discriminating sensitive tasters from non-

tasters (Lucchina *et al*., 1998). In addition, Psychophysical taste tests using the LMS revealed particular differences in taste sensitivity and perception associated with taster status (Bartoshuk *et al.,* 2004).

1.2.5.3.3 Threshold measurements

Sensory thresholds are a measure of human sensitivity to a given stimulus. They are an essential element in sensory analysis (Meilgaard *et al*., 1999). Thresholds are the limits of sensory capacities. They are distinguished between the detection threshold, the recognition threshold, the difference threshold and the terminal threshold. Different sensory thresholds have been defined (Lawless and Heymann, 2010) as following.

- Detection or absolute threshold is the lowest level at which a stimulus can be detected.
- Recognition threshold is the level at which a stimulus can not only be detected but also recognized. The recognition threshold is usually higher than the detection threshold.
- Differential threshold is the level at which an increase in a detected stimulus can be perceived. It is the extent of change in the stimulus necessary to produce a noticeable difference.
- Terminal threshold is the level beyond which a stimulus is no longer detected or there is no increase in the perceived intensity.

In food research, the recognition threshold for a given flavour in a food would be a useful thing to know and perhaps more useful than detection thresholds, since both the percept and the appropriate label have been made consciously available and actionable to the taster. In the case of off flavours or taints, recognition may have strong hedonic correlates in predicting consumer rejection (Lawless and Hayman, 2010).

A sensory threshold testing standard utilizes a presentation method called 3-alternative forced-choice (3-AFC) or ascending forced-choice. This method aims to determine a practical value close to the threshold, based on a minimum of testing effort. It makes an approximate best estimate determination of each threshold panellist (Meilgaard *et al*., 1999). The 3-AFC is a sample presentation in which three samples are presented; two are controls and one contains the target sample (substance

under test). The panellists must select the one of the three that is different from the other two. The panellists were required (forced) to choose one of the three and acknowledge their response as a guess, detection or recognition, according to ASTM E679-04. After the first set of presentations, the panellists were then presented with the next dilution level. At this next level, the panellists were again presented with three sample choices, one of which is the diluted of substance under test. However, this next dilution level presents the substance at a higher concentration (i.e. two times higher). The panellists proceed to evaluate higher levels of substance, following these methods until the substance concentration is above the recognition threshold. Results are computed for each panellist based on the dilution level where the correct threshold responses were recorded (St. Croix Sensory, 2007). The best estimate threshold (BET) for each panellist is the geometric mean of the highest concentration missed and the next higher concentration. The group of BET is the geometric mean of the individual BETs (Meilgaard *et al*., 1999).

Threshold determination has been used to measure human perception for pungency. Lawless *et al.* (2000) evaluated the hotness threshold degree of capsaicin in oil- and water-based model systems by user and non-user groups. Thresholds were measured among 23 panellists using an ascending forced choice method of limits. In water-based carriers, concentrations of capsaicin in water for threshold testing were 0.03125, 0.0625, 0.125, 0.25, 0.5 mg/l and 0.316, 1.0, 3.16 and 10 mg/l for the scaling study. In the oil-based carriers, concentrations of capsaicin for threshold testing were 1, 2, 4, 8 and 16 mg/l in vegetable oil for thresholds and 10, 32, 100 and 316 mg/l in vegetable oil (100% soybean) and corn oil for the scaling study. ASTM procedure E679 (2004), an ascending alternative forced-choice method of limits (3-AFC), was used to measure threshold. Ratings were made on 15 cm horizontal labelled magnitude scale. It was found that detection thresholds were 11.75 mg/l in oil and 0.31 mg/l in water. This is due to capsaicin is a lipophilic and thus more readily soluble in lipids, resulting overestimate the perceived hotness intensity in a fat containing food. The differences between user and non-user groups of spicy foods were less pronounced in water-based stimuli ($P>0.05$). However, non-user groups of spicy foods had higher thresholds and higher suprathreshold responses in oil systems.

1.2.5.3.4 Consumer liking test

Liking test is a quantitative affective test which determines the responses of consumers (Meilgaard *et al*., 1999). A large number (50 to several hundreds) of consumers representing the general public must be used (Vaclavik and Christian, 2003; Meilgaard *et al*., 1999). Consumers, who are not the trained panellists, are used for this type of sensory testing. They give their opinions regarding to the samples (Lawless and Heymaan, 2010). However, they are normally screened to make sure that they are users of the product to be tested (Meilgaard *et al*., 1999). Typically, liking test is used when a product's researcher needs to determine an affective status of a product, i.e. how well it is liked by consumers. The product is compared to a well liked company product or that of a competitor. A hedonic scale is used to indicate the degree of unacceptable or dislike to like. The relative acceptance scores can imply to liking, the sample with the higher score is liked. The best results are obtained with scales that are balanced (Meilgaard *et al*., 1999). The most common liking scale is a 9-point hedonic scale. The hedonic scale has achieved wide popularity. It is assumed that consumer likings exist on a continuum and that likings can categorised by responses based on likes and dislikes. The consumers are asked to evaluate a sample and score it on the 9-point hedonic scale from dislike extremely or like extremely (Vaclavik and Christian, 2003). The spacing on the hedonic scale is equal-interval, which is important in the assignment of numerical values to the response choices and to the use of parametric statistics in analysis of the data. The 9-point hedonic scale is very simple to use and is easy to implement. The hedonic scale is reliable and has a high stability of response that is independent of region and to some extent of panellist size (Lawless and Heymaan, 2010). It is decided that it fitted better on the typing paper by Chicago and the Quarter master Institute (Lawless and Heymaan, 2010). Jaeger *et al*. (2008) suggested that it is a direct scale of hedonic magnitude, because the consumers directly assess their hedonic experience by assigning it to one of the nine discrete categories, which represent differences along the liking/ disliking dimension. Pearce *et al*. (1986) compared the liking score of eight fabrics using three scales of 9-point hedonic, unstructured line scales and magnitude estimate scales. It was found that data from the three scales were similar in terms of reliability, precision and discrimination. An advantage of the 9-point hedonic scale is

possibility to convert the hedonic scale results to other affective data, such as paired preference or ranking data (Rohm and Raaber, 1991). Additionally, the hedonic data can be used in preference mapping technique (Greenhoff and MacFie, 1994; Helgesen *et al*., 1997). This is very valuable procedure that allows visualisation of directions for product preference in spatial models of a product set. In spatial model from multivariate analysis, products are represented by points in the space. The products, which are similar, are positioned close together. Dimensions or attributes, which differentiate the products, are then inferred from their positions in the space from opposites positioned at different sides and from interpretation of axes of the space. In one form of preference mapping, the preferences of consumers are projected as vectors through the space to show directions of increased liking. These vectors can then suggest direction for product optimization. In addition, different in the preferred directions of different consumers can suggest market segments or groups with different likes and dislikes (Lawless and Heymaan, 2010).

1.3 Scope of research

There are 4 parts in this study, Part 1, the effect of three drying processes, i.e. sun, hot air and freeze drying on volatile flavour compounds by GC-MS and capsaicin content by HPLC will be studied as well as the physical and chemical qualities of dried Chee fah chilli. Part 2, the sensory profile of dried Chee fah chilli will be developed by trained panellists. In addition, the correlation between instrument results and trained panellists on the volatile flavour compound and capsaicin contents which responsible for pungent odour and hotness of dried Chee fah chilli will be explored. Part 3, the difference in sensory threshold of the pungent odour and hotness perceived by light, moderate and heavy user will be evaluated. Finally, part 4, consumer liking of hotness and pungent odour intensities on processed dry Chee fah chilli will be observed.

CHAPTER 2

EFFECT OF DRYING METHOD ON PHYSICAL AND CHEMICAL QUALITY, HOTNESS AND VOLATILE FLAVOUR CHARACTERISTICS OF CHEE FAH CHILLI

2.1 Abstract

The effects of drying methods, such as sun drying, hot air drying at 60^{o}C and freeze drying, on the quality of dried Chee fah chilli (*Capsicum annuum* Linn. var. *Acuminatum Fingarh*.) were investigated. The quality parameters such as moisture content, colour (L*, a*, b* values), ascorbic acid content, capsaicin content and volatile flavour were mentioned. The freeze dried (FD) sample gave more bright-red colour and contained higher ascorbic acid content than the sun dried (SD) and hot air dried (HD) samples ($P<0.05$). Meanwhile, moisture content (11%) and capsaicin content (~1 ppm) were not significantly different among the three drying methods ($P>0.05$). Types and concentrations of volatile flavour compounds were detected using headspace solid phase microextraction (HS-SPME) and liquid-liquid extraction (LLE) with a gas chromatography-mass spectrometry (GC-MS). The groups of volatile flavour compounds were acids, alcohols, ketones, aldehydes, esters, furans and hydrocarbons.

2.2 Introduction

Dried chilli is a spice product and the one most widely used as condiments for flavouring and colouring in Asian cuisines (Jitbunjerdkul and Kijroongrojana, 2007; Toontom *et al*., 2010). The quality of dried chilli is assessed by a number of different parameters such as colour, hotness, ascorbic acid content and volatile flavour compounds (Henderson, 1992; Ruth *et al*., 2003; Jiang and Kubota, 2004, Kim *et al*., 2006; Wang *et al*., 2009; Yaldiza *et al.,* 2010). Traditionally, dried chilli is obtained by sun drying (Condori *et al*., 2001; Oztekin *et al.,* 1999). It takes about 7-20 days (depending on the weather conditions) to reduce the moisture content to 10-15% (Oberoi *et al.*, 2005; Hossain, 2003). Since dried chilli is susceptible to fungal proliferation, this process creates favourable conditions for mycotoxins

contamination (Bircan, 2005). To prevent fungal proliferation, different drying methods have been employed in the processing of dried chilli.

Currently, hot air drying is popular for drying chilli due to a relatively short drying time, uniform heating and more hygienic characteristics. The temperature ranges from 45 to 70^{o}C (approximately 10% of moisture content), and this reduces drying time to less than 20 hrs. This temperature range gives maximum colour values and minimizes the loss of volatile oils and discolouration (Mínguez-Mosquera *et al*., 1994; Díaz-Maroto *et al.*, 2003; Ibrahim *et al.*, 1997; Berke and Shieh, 2001). However, freeze drying is the best method of water removal as it gives a final product of the highest quality without heat compared to other methods of food drying (Genin and René, 1995; Irzyniec *et al.,* 1995). It has been found that this is the most suitable drying method for maintaining the colour quality of dried chilli (Park and Kim, 2007). However, the flavour formation may not meet the requirement of the consumers.

Solid-phase microextraction (SPME) has been recommended for the quantitative analysis of flavour and fragrance compounds (Zhang *et al*., 1994). This technique is based on a fused-silica fiber, coated with polymeric stationary phase, introduced into a liquid or gas sample. It involves two processes; partitioning of analytes between the coating and the sample, and thermal desorption of analytes into gas chromatograph (Ibanez *et al.*, 1998; Solis-Solis *et al*., 2007). SPME is a rapid and simple procedure for extraction of volatile fraction from aromatic plants (Paolini *et al*., 2008; Belliardo *et al*., 2006; Mazisa *et al*., 2005). However, extraction by SPME depends on the volatility and adsorptivity to SPME fiber of the volatile compounds (Lee *et al*., 2012). In addition, the SPME volatile concentrations are relatively low and thus difficult to analyze by MS when splitting the sample with a GC column (Mahattanatawee *et al*., 2005). Therefore, only SPME may not success to get a good representation of the volatile compound in a sample. Many researchers recommended application of SPME extraction combined with liquid-liquid extraction (LLE) to obtain a more complete volatile flavour profile of samples (Mahattanatawee *et al*., 2005; Solis-Solis *et al*., 2007; Kuwayama *et al*., 2008; Domínguez and Agosin, 2010; Lee *et al*., 2012). Since, extraction by SPME method mainly depends on the volatility and adsorptivity to SPME fiber of the volatile flavour compounds and LLE depends on the pKa and solubility in extraction solvent. Therefore, combination of usage the

both method can improve reliability of the profiling results (Lee *et al*., 2012). The object of this work is to study the effects of drying methods on the quality, hotness and volatile flavour characteristic of dried chilli.

2.3 Materials and Method

2.3.1 Chemicals

Capsaicin (≥95.0%, from *Capsaicum* sp.) and 1-penten-3-one (1P3O) (97.0%) were purchased from Sigma-Aldrich Co. LLC. (USA). Methanol and water (HPLC grade) were obtained from Prolabo (Paris, France). L-ascorbic, 2,6-dichlorophenol indohenol, sodium hydroxide, acetone, phosphoric acid and phenopthalein were obtained from Prolabo (Paris, France). Acetone, dichloromethane and sodium chloride were obtained from LabScan Asia Co., Ltd (Bangkok, Thailand). Pentane was purchased from BDH Laboratory Supplies (Poole, England). Sodium sulfate anhydrous was purchased from Fisher Scientific (Leicestershire, England).

2.3.2 Raw materials and sample preparation

Chee fah chilli (*Capsicum annuum* Linn. var. *Acuminatum Fingerh*.) was purchased from a contacted distributor from local market in Songkhla province. Fully ripened chilli (70-90 days at the time of harvesting) with red colour of at least 75% of surface pod was used. The average diameter of pods was 1.5 ± 0.24 cm and an average length was 10.4 ± 0.98 cm. The raw material specifications in this research were based on Thai Agricultural Commodity and Food Standards of fresh chilli (TACFS 1502-2004). One hundred kilograms of the chilli pods were mixed by hand to provide homogeneity of the original raw material batch. The batch then was equally divided into 4 random batches for the control sample (fresh chilli) and for processing batches of the three drying treatments. For control treatment, the fresh chilli was immediately washed, removed the stem and prepared to further analyses, immediately. For drying treatments, the whole pod of fresh chilli was washed, removed the stem, blanched using hot water at 90^{o}C for 3 minutes (Gupta *et al.*, 2002), and then cooled in cold water and drained on a perforated tray. The chilli was cut into approximately 2 cm lengths before drying.

2.3.3 Chilli drying with different methods

Sample obtained from 2.3.2 was used in this experiment. Three different drying methods; hot air drying (HD); freeze drying (FD); and sun drying (SD) were used. The fresh (F) Chee fah chilli without drying was used as a control (Appendix 1, Figure 1). SD was conducted by spreading blanched-cut chilli on a net in a single layer and exposed directly to sunlight (approximately 37^{o}C). The thermometer was placed on an empty tray besides a net of chilli. This method was dried for 8 hrs per day. A temperature of 60^{o}C was used for the HD. The blanched-cut chilli was placed on perforated tray which has an area of approximately 0.2 m^{2}. Freeze-drying chilli was performed at -50^{o}C, 5 Pa in a freeze dryer. All the dried chilli samples were taken when the moisture content was approximately 10-13% (According to the Thai Industrial Standards Institute: TISI 456-1983) (Appendix 1, Figure 2). The individual final product from each drying treatment was packed together as a whole lot in aluminium laminated bags under vacuum condition and stored at -20^{o}C. Before analysis, the sample was ground, passed to sieve (80 meshes) and then subjected to analysis (Appendix 1, Figure 3).

2.3.4 Measurement of physical and chemical qualities

2.3.4.1 Determination of moisture content and water activity (a_w)

The A.O.A.C method (A.O.A.C, 2000) was used for determining the moisture content using a hot air oven at a temperature of 105^{o}C. Water activity was measured using a water activity meter (Novasina, Thermostanter) calibrated as a standard sample with a known value (Range 0.11-0.99). The measurements were taken in triplicate and results were averaged.

2.3.4.2 Colour measurements

Surface colour of the chilli was measured in CIE system on L* (lightness), a*(redness and greenness) and b*(yellowness and blueness), using a Hunter Lab Colourflex colourimeter. All samples were cut lengthwise, faced down and spread out to evaluate for colour. The L*, a* and b* measurements were then calculated into hue angle (given by the equation $\tan^{-1}b^*/a^*$) and chroma (given by $\sqrt{a^{*2} + b^{*2}}$) in order to provide more practical interpretation of colour (McGuire, 1992). The total colour difference (ΔE^*) of dried chilli samples were calculated by

$\Delta E^* = \sqrt{(L^*-L_0) + (a^*-a_0)+ (b^*-b_0)}$, where L_0, a_0 and b_0 are the control values for fresh chilli (Sigge *et al.,* 2001). There were three replications of measurement in each treatment, using 10 chilli pods per replication (Wiriya *et al*., 2009; Topuz *et al*., 2009; Chaethong *et al.,* 2012). Hence the colour data from thirty measurements was averaged.

2.3.4.3 Determination of pH

Five grams of Chee fah chilli ground sample was diluted with 10 ml of distilled water. It was measured for the pH value at ambient temperature with a pH meter (Satorious, USA) which was calibrated with pH 4.0 and 7.0 (A.O.A.C, 2000). The measurements were taken in triplicate and results were averaged.

2.3.4.4 Determination of total acidity

Chee fah chilli ground samples were diluted with 10 ml of distilled water and titrated with 5 N NaOH using a few drops of 1% phenolphthalein solution as an indicator. The sample was transferred as a measured quantity (10 g) into 10 ml of distilled water. The result was calculated as the percentage of citric acid (Adapted from Ranganna, 1986). The measurements were taken in triplicate and results were averaged.

2.3.4.5 Determination of ascorbic acid

Ten grams of Chee fah chilli ground sample were diluted with 10 ml of distilled water, filtered by a vacuum process, centrifuged with 15,000 rpm for 30 min and the supernatant was obtained. Five milliliters of extracted sample was added to 2,6-dichlorophenol-indophenol solution. The quantity of ascorbic acid in each sample was quantified by comparing against the standard of ascorbic acid. Standard solutions of ascorbic acid were prepared using 2% metaphosphoric acid. A standard curve was delivered by using serial dilutions of 0.00 (control), 0.20, 0.40, 0.60, 0.80 and 1.00 mg/ml ascorbic acid. Stock solutions contained 1.00 mg/ml ascorbic acid. A pipette was used to measure the requisite volume of standard ascorbic acid solutions of 1, 2, 3, 4 and 5 ml. These were made up to 5 ml with the requisite amount of 2% metaphosphoric acid. Ten milliliters of 2,6-dichlorophenol-indophenol solution was added using a rapid delivery pipette, then mixed and taken for the determination within 15-20 s. The instrument was set to 100% transmission using a blank consisting

of 5 ml of 2% metaphosphoric acid solution and 10 ml of water. Light-absorption was measurement at 518 nm and the result was then calculated by deduction of absorbance of sample from the control (Sroka and Cisowski, 2005). The obtained absorbance was plotted against concentration (Ranganna, 1986) (Appendix 2, Figure 7 and Table 1). The measurements were taken in triplicate and results were averaged.

2.3.4.6 Determination of capsaicin content

2.3.4.6.1 Extraction of capsaicin from fresh and dried chilli

Ten grams of Chee fah chilli ground sample was placed in a 250 ml flask with 100 ml of acetone. The sample was stirred for 1 hr at room temperature (Appendix 1, Figure 4). It was filtered by vacuum and the volume of the supernatant was reduced to approximately 5 ml by removing the excess acetone using nitrogen gas. The final solution was filtered through a 0.45 mm filter before injection to high performance liquid chromatography (HPLC).

2.3.4.6.2 High performance liquid chromatography analysis

Ten microliters of extracted sample was injected for analysis by HPLC equipped with a Luna C18 column (5μ, 250 × 4.6 cm) and a UV detector at 284 nm. The mobile phase used a mixture of methanol and water (80:20 v/v) and a flow rate of 1.5 ml/min (Betts, 1999). The capsaicin in each sample was identified and quantified by comparing it with capsaicin standard compounds (≥95.0%, from *Capsaicum* sp., Sigma, USA). A standard curve was prepared using serial dilutions of 0.15, 0.31, 0.63, 1.25, 2.50, 5.00, 10.00 and 20.00 mg/l capsaicin concentrations. The pungency level in SHU was calculated by using the amount of capsaicin (%dry weight) x 160,000 (Govindarajan, 1986). The measurements were taken in triplicate and results were averaged.

2.3.4.7 Volatile flavour compounds analysis

2.3.4.7.1 Sample extraction

Liquid-liquid extraction (LLE): Forty grams of ground sample were mixed with 200 ml of distilled water, filtered by vacuum, centrifuged with 1500 rpm for 10 min at 4ºC (Nabavi *et al*., 2010) and removed the supernatant to use for determination. Then, clear solution was used for volatile flavour compounds isolation, immediately. The 100 ml clear solution was mixed with 100 ml solvent

(dichloromethane and pentane; 1:2 v/v). The extraction was performed at room temperature by using magnetic stirrer. Then, the mixture was extracted for 90 min and left equilibrates for 30 min. Solvent phase was collected and sediment was re-extracted twice. The combined solvent phase was dried on sodium sulfate anhydrous, kept overnight at -20°C, cold- filtered and concentrated by purging of nitrogen gas. The concentrate was kept at -20°C prior to analysis. The 1 µl was directly injected into GC-MS.

Solid phase micro extraction (SPME): Three grams of ground sample ware adsorbed onto a solid phase microextraction (SPME; Supelco Inc., Bellefonte, PA) holder for GC analysis using the MS detector. Each SPME sampler consisted of a length of fused silica fibre absorption fibres coated with divinylbenzene, carboxen and polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm as a solid adsorbent.

The sample was taken for sampling in a glass vial of 20 ml capacity and capped with a Teflon-lined septum and crimped. The vial was incubated at 40°C for 20 min in the heating block chamber before the introduction of fibre into the headspace vial. The volatile flavour was adsorbed at 40°C for 20 min and subsequently thermally desorbed at 220°C for 5 min in a GC injection port. Desorption time was optimized to ensuring there would be no carry-over effect to the next sampling.

2.3.4.7.2 Gas chromatography–mass spectrometry

Volatile flavour compounds were identified using Gas Chromatography-Mass Spectrometry (GC-MS) using an Agilent 6890 plus GC/HP 5973 MSD (Agilent, USA). The carrier gas was helium at a flow rate of 1.5 ml/ min with a split ratio of 1:1 at 220°C. The separation of volatile flavour compounds was achieved on a fused silica capillary column (25 m x 0.32 mm i.d.) coated with crosslinked polyethylene glycol modified with nitroterephthalic acid as a stationary phase (20 M) at a film thickness of 0.50 µm (HP-FFAP; J&W Scientific, Folsom, CA). The oven was programmed as follows: 45°C for 2 min, ramped to 130°C at 3°C/min and held for 1 min; ramped to 220°C for 3 min at 20°C/min; and then ramped to 230°C for 1 min at the same rate. The mass selective detector capillary direct-interface temperature was 280°C. Acquisition was performed in the electronic

impact (EI) mode. The mass range used was 20-550 a.m.u. and the acquisition rate was 4.33 scan/sec. The identification was tentatively based on a comparison of the mass spectra of unknown compounds with those in the Wiley 275.L mass spectral database (Hewlett-Packard Co.) (Toontom, 2008).

2.3.5 Statistical analysis

A Completely Randomized Design (CRD) was planned for this experiment. Data were subjected to analysis of variance (ANOVA). Significant differences between means were estimated by Duncan's new multiple range test (DMRT), with a level of significance of 0.05. Statistical analyses were performed by using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA, 2002). Principal Component Analysis (PCA) was applied to observe any relationships among the capsaicin contents, the physical and chemical qualities of fresh and dried chilli using different drying methods were assessed by XLSTAT software (XLSTAT Pro 2008).

2.4 Results and Discussion

2.4.1 Effect of drying on physical and chemical qualities

The physical and chemical qualities of all the chilli dried with different drying methods were compared to the fresh chilli as shown in Table 5.

Table 5 Physical and chemical qualities of fresh and different drying samples

Physical and chemical qualities		**F**	**FD**	**HD**	**SD**
Colour	L*	38.34±1.18^{a}	37.41±0.06^{a}	31.45±0.65^{c}	32.95±0.68^{b}
	a*	34.31±0.85^{a}	29.56±0.07^{b}	20.65±0.61^{c}	10.83±0.55^{d}
	b*	15.89±0.64^{b}	25.72±0.17^{a}	14.40±0.20^{c}	4.61±0.21^{d}
	Chroma	37.95±0.47^{a}	38.76±0.85^{a}	25.17±0.70^{b}	11.86±0.42^{c}
	Hue angle	25.31±0.47^{c}	40.28±0.73^{a}	34.89±0.48^{b}	22.87±0.67^{d}
	ΔE*	-	10.16±0.73^{c}	15.71±0.56^{b}	26.77±0.39^{b}
Moisture content (%wb)		85.15±0.74^{a}	11.16±0.21^{b}	11.06±0.06^{b}	11.07±0.36^{b}
Water activity		0.99±0.11^{a}	0.68±0.03^{b}	0.59±0.04^{b}	0.65±0.01^{b}
pH		5.62±0.10^{a}	4.84±0.25^{b}	4.67±0.14^{c}	3.21±0.12^{d}
Total acidity (% db)		0.06±0.03^{d}	0.15±0.01^{c}	0.23±0.01^{b}	0.59±0.03^{a}
Ascorbic acid content (mg/100g db)		53.18±1.50^{a}	51.55±0.54^{a}	28.34±0.94^{b}	14.21±0.72^{c}

Note: Different superscripts within a row show significant difference (P≤0.05).

ΔE* refers to total colour difference.

The initial average moisture content and water activity of fresh chilli were 85.15% and 0.99, respectively. The average moisture contents of all dried chilli

were 11% wb and water activities varied between 0.51 and 0.68. The moisture content of chilli is very important because it is strongly correlated with the stability of ascorbic acid and pigment as well as any hygiene problems (Kim *et al*., 1982). Carbonell *et al*. (1986), Lee *et al*. (1992) and Kanner *et al.* (1977) reported that the moisture content of dried chilli ranged from 10 to 14% which could retard colour loss and the moisture content lower than 8% could accelerate pigment destruction. Wall and Bosland (1993) reported that final moisture content at 8% is ideal. Moisture content above 11% allows mould to grow and moisture content below 4% causes an excessive colour loss. However, chilli generally needs to be dried to a moisture content of below 13% in order to prevent potential aflatoxin production (Pitt and Hocking, 1997). This is also recommended for Thai dried chilli as regulated by the Thai Industrial Standards Institute (TISI 456-1983).

The different drying methods affected on the colour qualities of chilli. Lightness (L*), redness (a*) and yellowness (b*) were significantly different among the samples ($P \leq 0.05$). It was shown that the L* values of all dried chilli ranged from 31.45 to 37.41. The a* values ranged from 10.83 to 29.56 and the b* values ranges from 4.61 to 25.72. Compared with the fresh chilli (L*= 38.34 and a*= 34.31), the FD sample was more similar in L* and a* values than the other drying methods ($P>0.05$). The colour of F sample was comparable to the colour of fresh *Capsicum annuum* reported by Cervantes-Paz *et al*. (2012) and Topuz *et al*. (2009) of L* (33.3-36.27), a* (29.56-32.62) and b* (14.47-28.12). In addition, the colour of FD samples of the present study were in the same range of freeze-dried *Capsicum annuum* samples (L* =39.14, a*=32.10 and b*=25.67) reported by Topuz *et al*. (2009). Whereas, the colour of sun dried chilli (*Capsicum annuum*) sample reported by Wiriya *et al*. (2009) was quite darker (L* = 23, hue angles = 36 and chroma = 29) than the colour of SD sample presented in this study. Since, the range of drying temperature from Wiriya *et al*. (2009) (26-53^{o}C) were higher than the range of drying temperature in this study (24-45^{o}C). The hue angle and chroma aspects of colour are easier to conceptualise than a* and b* values. All dried chilli samples presented hue angles of 22.87-40.28

and chroma of 11.86-37.95. The colour of F sample could be described in bright orange-red, FD in bright reddish-orange, and HD in dull orangey-red. Whereas, SD colour could be described as very dull red, as shown in Appendix 1 (Figures 1-2). The hue angles of FD and HD were in commercially acceptable ranges which are between 35 and 45 (Osuna-Garcia and Wall, 1998). The total colour difference (ΔE^*) of all dried chilli samples was calculated using colour of fresh chilli as the reference. A larger value of ΔE^* indicates greater colour change from a reference material. The result reveals the colour change in dried chilli from fresh chilli was influenced by the drying method ($P \leq 0.05$). The ΔE^* values extremely decreased on sun drying sample. This indicates that the freeze drying method significantly improved the lightness and redness of dried chilli compared to the other drying methods ($P \leq 0.05$). The minimal colour deterioration during the freeze drying is an indication of the appropriateness of this method to preserve nutraceutical foods (Ratti, 2001). On the other hand, non-enzymatic browning is another cause of chilli colour degradation in the HD sample. This was because the heat temperature (60^oC) and long time (8 hrs.) provide in this method is used to achieve the required moisture level in the dried chilli. It may be also related to the concentrations of sugar and amino acid in the chilli. It has been reported that non-enzymatic browning in dried chilli is due to a maillard reaction between reducing sugar and amino acid in pericarp (Lee *et al*., 1991). It is expected that the browning reactions will be minimized by the low temperature used in the freeze dried method. Hence the FD sample showed less colour deterioration than the HD sample. However, higher colour degradation in the SD sample was due to pigment oxidation and decomposition during exposure to oxygen when an intensive vaporization took place on the surface of this chilli (Topuz and Ozdemir, 2004).

The pH and total acidity of dried chilli were significantly different among the samples ($P \leq 0.05$). The pH value of all dried chilli varied between 3.21 and 4.84, while the total acidity was found to be in a range from 0.15 to 0.59%. The SD sample was lower in pH and higher in total acidity values than the FD and HD

samples. Fresh chilli had the highest pH and was the least total acidity values. This result was not in agreement with the study undertaken by Wiriya *et al*., (2009) who reported that sun dried chilli had lower total acidity than fresh sample. There might be influences of varied conditions during sun drying (i.e. time, temperature and relative humidity), which depended on the weather conditions (Condori *et al*., 2001). The pH and total acidity may be mainly dominated by citric acid which is the main organic acid present in chilli (Koh, 2005). However, variations of pH and total acidity may be caused by contamination from microorganisms. Microorganisms, mainly lactic acid bacteria, produce organic acids, which then increase in total acidity content and decrease in pH value. Generally, sun dried chilli becomes more contaminated with microorganisms than in the other drying processes (Mangaraj *et al.*, 2001). Hence, these variations in pH and total acidity can be used to indicate the safety of food.

The ascorbic acid contents of all of dried chilli were significantly different among the samples ($P \leq 0.05$). The ascorbic acid contents of all dried chilli varied between 14.21 and 51.55 mg/100g, whereas, the ascorbic acid content of fresh chilli was 53.19 mg/100g. However, the FD sample had higher ascorbic acid content than the HD and SD samples ($P \leq 0.05$). This result agreed with Howard *et al.* (1994) who studied ascorbic acid content of fresh chilli cultivars (*Capsicum annuum*) and found that the ascorbic acid of red chilli decreased during drying. Howard *et al.* (1994) also reported that 75% of ascorbic acid in red chilli was lost during drying, with the final content of ascorbic acid being in a range from 12.0 to 44.4 mg/100 g. Vega-Gálvez *et al*. (2008) supported that temperature in the HD method had a detrimental effect on the retention of ascorbic acid. Likewise, Veras *et al.* (2012) found that the chilli (*Capsicum baccatum*) dried by freeze drying method had higher levels of ascorbic acid content than the sample produced by hot air drying (50-70^{o}C) method. They also reported that the losses during freeze drying were around 43.7% with respect to in fresh chilli samples. This is because low temperatures of freeze

drying and the absence of dry air prevent the degradation of ascorbic acid (Veras *et al.*, 2012). Whereas, the heated air inherently exposes the products to oxidation, thus reducing their ascorbic acid content. Ascorbic acid is oxidized by light and high temperature during drying leading to the formation of L-dehydroascorbic acid and a wide variety of carbonyl and other unsaturated compounds (Gregory, 1996; BeMiller and Whistler, 1996). According to the Food Composition Table (RDA, 2001), the ascorbic acid content of dried chilli is about 26 mg/100 g (Kim *et al.*, 2006). This is a lower content than in the present research, except for the SD sample. This means that ascorbic acid has been destroyed less in our drying methods and high ascorbic acid is contained in all the dried chilli, especially in the FD sample.

2.4.2 Effect of drying on capsaicin content

The HPLC chromatograms shown in Figures 5 correspond to fresh and dried chilli samples as well as the standard of capsaicin. They reveal that the capsaicin was eluted at 4.21 min and the dihydrocapsaicin was eluted at 5.40 min with different amount.

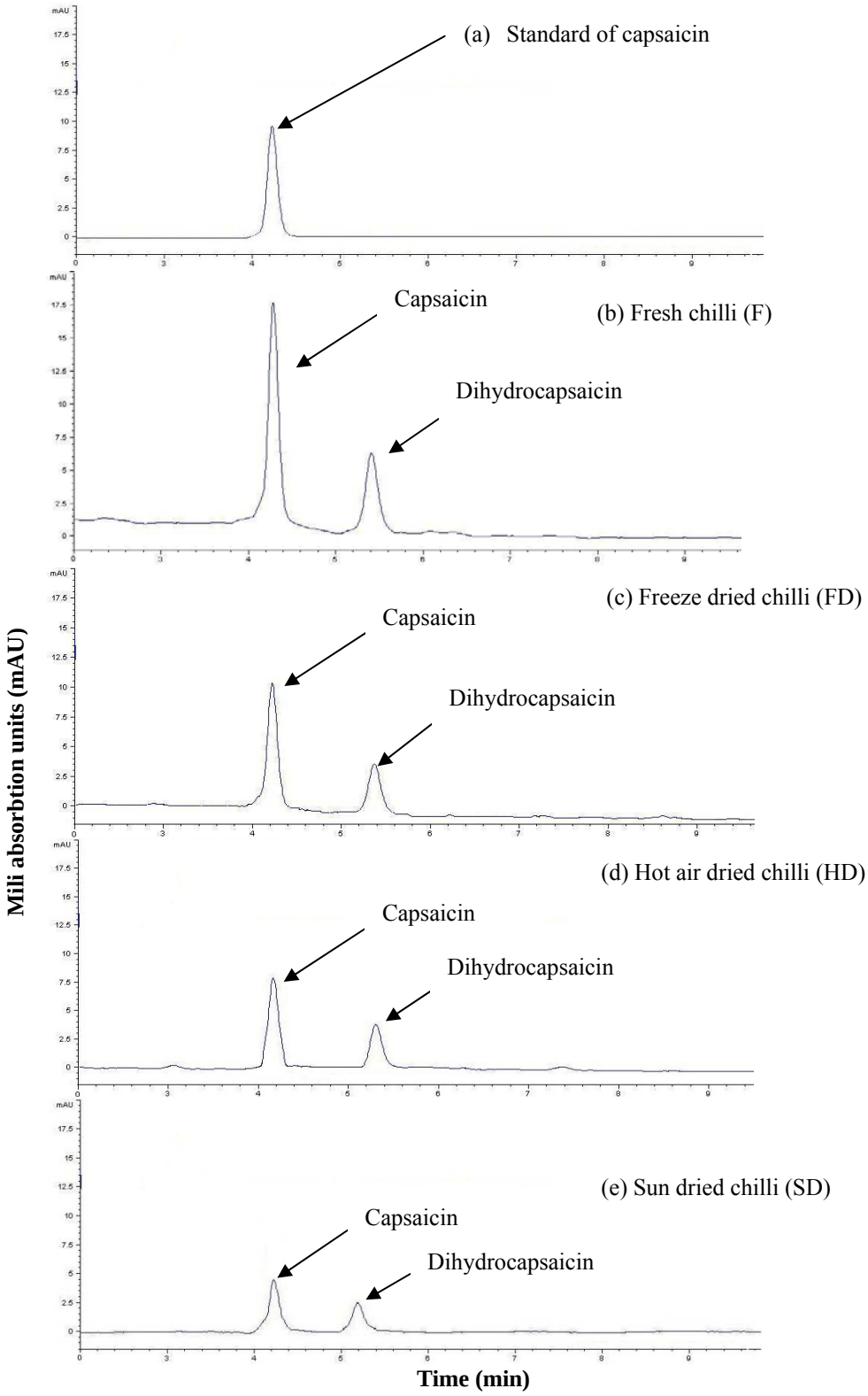


Figure 5 HPLC chromatograms of standard of capsaicin (a), capsaicin in fresh chilli (b) and samples with different drying methods (c-e)

Figure 6 shows the capsaicin contents of three dried samples which were calculated based on weight dry basis. It was found that the drying method did not affect the capsaicin content (P>0.05). Furthermore, the capsaicin content and hotness of dried chilli from all the drying methods were higher than in the fresh chilli sample (P≤0.05).

Table 6 Capsaicin and hotness level of fresh and dried chilli using different drying

Samples	Capsaicin contents (mg/g db)	Hotness levels (SHU)
F	0.58 ± 0.10^{b}	9280.84 ± 705.71^{b}
FD	1.29 ± 0.20^{a}	20640.54 ± 743.87^{a}
HD	1.17 ± 0.20^{a}	18720.20 ± 335.46^{a}
SD	0.98 ± 0.21^{a}	15680.79 ± 650.60^{a}

Note: Different superscripts within a column show significant difference (P≤0.05).

The capsaicin content of fresh chilli in this research was in a range of fresh Chee fah chilli (0.52-1.07 mg/g; 7,500-16,500 SHU) that reported by Kaewprasit and Kumngern (2009) and Noichinda *et al*. (2012). However, the fresh chilli showed lower capsaicin content than the three dried chilli samples which could be due to the inactivation of peroxidase enzyme. Bernal *et al*., (1993a, 1993b) suggested that vanillyl moiety of capsaicin was easily oxidized by the peroxidise enzyme. This enzyme could contribute to capsaicin degradation. Whereas, dried chilli samples were blanched before in order to inactivate this enzyme before drying. The similar result was reported by Schweiggert *et al.* (2006) and Topuz *et al.* (2011). The capsaicin contents of all dried chilli varied between 0.98 and 1.29 mg/g (P>0.05). The hotness levels of all dried chilli varied between 15,680.79 and 20,640.54 SHU (P>0.05). Yaldiza *et al.* (2010) also reported that the capsaicin content of dried chilli (*Capsicum frutescens*) varied between 0.50 and 4.20%. This was due to temperature, time and drying methods. Topuz and Ozdemir (2004) reported that sun dried Turkish paprika chilli, which was processed for 5-7 days, lost 24.6% of the capsaicin content (approximately 12-14% moisture content). Oven-dried Turkish paprika chilli, which was dehydrated at 70^{o}C for 90 min, lost 21.5% of the capsaicin content. On the other hand, thermally-treating chilli at 210^{o}C was reported to increase the capsaicin content

(6.1-924.9%). This was caused by the dehydration of the food matrix and improved extractability of capsaicin by cell disruption during the thermal process (Harrison and Harris, 1985; Lee and Howard, 1999; Schweiggert *et al.,* 2006).

PCA is normally used to illustrate the relationships among all qualities and the grouping of the samples is shown in Figure 6. The PCA is composed of two Principal Components (PC) that indicate 91.48% of the variability of the data. Capsaicin content and hotness are presented as having a highly positive correlation to the HD sample. On the other hand, colour qualities and ascorbic acid content are presented as having a positive correlation to the FD sample, but a negative correlation to the SD sample. From the PCA, it can be seen that the HD sample shows the highest quality in terms of capsaicin content and hotness. On the other hand, the FD sample shows the highest quality in terms of colour and ascorbic acid content, whereas the SD sample shows the least in all qualities. This may be because the SD sample exposed to the air during drying for a long time (Mangaraj *et al.*, 2001; Daood *et al.*, 1996).

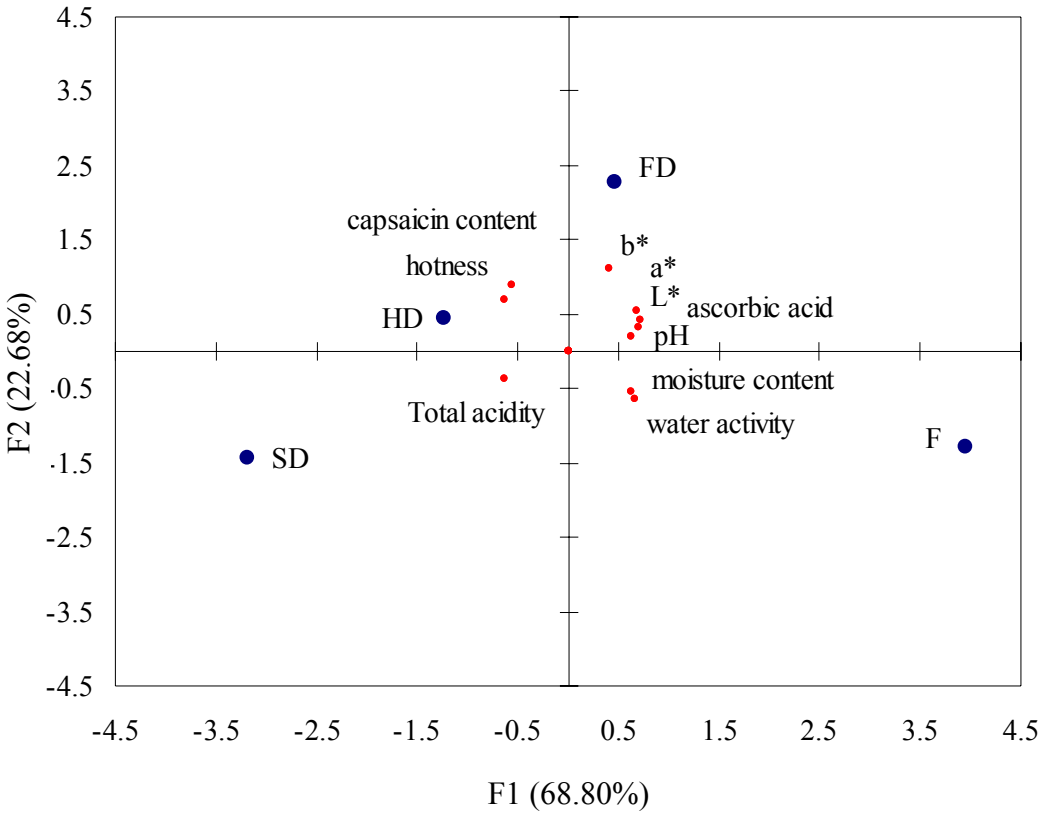


Figure 6 PCA bi-plots of physical and chemical qualities of fresh and dried chilli using different drying methods

2.4.3 Effect of drying on volatile flavour

The volatile fractions of fresh and dried chilli extracted using SPME and LLE techniques are shown in Table 7 (Appendix 2, Figure 5-6). From a qualitative viewpoint, volatile compounds namely; acids, alcohols, ketones, aldehydes, esters, pyrroles, sulphur and hydrocarbon were observed using LLE and SPME. However, the number of components obtained by the SPME was less than those extracted using LLE. Using SPME, the identification of 48 volatile components (4 acids, 9 alcohols, 4 ketones, 2 furans, 4 esters, 2 pyrroles, 8 aldehydes, 1 sulphur compound and 14 hydrocarbons) were performed by GC-MS. The result was revealed that higher amount of several alcohol, aldehyde and hydrocarbon compounds were found in SPME profiles. This may be due to the intermediate polarity of divinylbenzene/carboxen/ polydimethylsiloxane (CAR/DVB/PDMS) coating (Junior *et al*., 2011). Whereas, LLE provided the identification of 53 volatile components (11 acids, 8 alcohols, 8 ketones, 5 esters, 1 pyrroles, 5 aldehydes, 1 sulphur compound and 13 hydrocabons). More compounds of acids, ketone and hydrocarbon were presented in the LLE profiles. In addition, 1-penten-3-one (pungent odour) was only analysed and detected by LLE/GC-MS. The result indicates that the SPME is not able to extract the volatile flavour component 1-penten-3-one (1P3O) which is a principle of pungent odour compound found in chilli. It is possible that 1P3O has low volatility, but it solubilises in organic mixed solvent of dichloromethane and pentane. The mixed solvent has high extraction efficiency for a wide range of non-polar to polar compounds (Martín-del-Campo *et al*., 2011; Popescu *et al*., 2011).

The discrepancy of LLE and SPME can be explained by the fact that compounds detected by SPME are mainly related to their volatility, while with LLE, the extraction of compounds mostly relies on its solubility in the organic phase (Lee *et al*., 2012). However both techniques are able to detect compounds that are known to be indicative chilli.

The effects of drying on volatile flavour chilli compounds could be distinguished as two groups, that was the compound decreased or disappeared or the compound increased or was formed. It was found that there were 2 compounds, namely 5-methyl-undecane and 2,3-dihydro-3,5-dihydroxy-6-methyl-4(4H)-pyranone

that appear in all the dried chilli, corresponding to herbal and caramel odours (Flavournet and human odour space, 2004; Almonds, 2009). The 1P3O (pungent odour) was a volatile compound of all samples which decreased after drying and was only extracted by LLE technique. Whereas, acetic acid was mainly presented in all samples and increased after drying, particularly in the SD sample. The volatile flavour compounds completely disappeared after drying. These were: cyclobutylbenzene (sweet odour); 4-vinyl-2-methoxy-phenol (clove-liked odour); phenol (acrid or tar-like odour); 2-methoxy-phenol (smoky odour); n-hexyl acetate (herbal odour); hexadecanal (cardboard-liked odour); 2-dococen-1-al (woody odour); 2-pentyl-furan (green-liked odour); hexanal (leafy odour); hexanol (herbal odour) and hexadecanoic acid (waxy odour).

The volatile flavour compounds were found in the FD sample. These were: 1,6-dimethyl-naphthalene (woody odour); hexadecane (mild waxy odour); 5-ethyl-undecane (herbal odour); 2-furanmethanol-acetate (horseradish-like odour); phenylethyl alcohol (fresh odour); 2-ethyl-1-hexanol (citrus-like odour); 5-methyl-2-(1-methyl) cyclohexanol (camphoraceous-liked odour); 2-octanol (spicy odour); and 2-methyl-butanoic acid (cheesy odour). Some volatile flavour compounds were only found in HD sample. These were: 1-methoxy-4-(1-propenyl)-benzene (aniseed-like odour); alpha-gurjunene (woody odour); undecane (herbal odour); 2-acetyl furan (balsamic-like odour); benzeneacetaldehyde (flora-like odour); 1;3-cyclohexadiene-1-carboxaldehyde (herbal odour); 5-methylfurfural (spicy odour); benzaldehyde (flora-like odour) and 2-methyl-butyric acid (cheesy odour).

On the other hand, the SD method decomposed highly volatile flavour compounds. The compounds found in SD sample were 2-methyl-tridecane (mild waxy odour); 2,3-butanediol (onion-like odour); dihydro-2(3H)-furanone (creamy odour); 1-methyl-1H-pyrrole (herbal odour); tetramethylpyrazine (nutty odour) and 2-methyl-propanoic acid (cheesy odour). The appearance of 2-methylpropionic and 2-methylbutyric acid may be due to Strecker degradation. Short chain fatty acids, namely, 2-methylpropionic and 2-methylbutyric acid, are probably formed upon further oxidation during drying (Luning *et al*., 1995). The formation of volatile

flavour compounds, namely 2-acetyl pyrrole and furfural, were only detected in the FD and HD samples due to a Maillard reaction. This was in agreement with the work of Apriyantono and Ames (1993) who monitored the formation of Maillard reactions in a model system of xylose-lysine.

Table 7 Volatile flavour compounds and their attributes indentified in fresh sample and dried chilli using different drying methods

RT[A]	RI[B]	Volatile flavour compound	Attributes[C]	Peak areas (%)							
				Liquid-liquid extraction/GC-MS				SPME/GC-MS			
				F	FD	HD	SD	F	FD	HD	SD
		Acids									
2.78	1081	2-Methyl-butanoic acid	Cheesy	nd	nd	nd	0.42	nd	1.63	nd	0.35
17.80	1596	Acetic acid	Vinegar-like	nd	nd	nd	1.32	0.02	4.72	2.37	10.71
20.93	1127	2-Methyl-propanoic acid	Cheesy	nd	nd	nd	2.44	nd	nd	nd	2.44
24.80	1662	2-Methyl-butyric acid	Cheesy	nd	nd	0.63	nd	nd	nd	0.54	nd
23.73	1652	Propanoic acid	Rancid, Sour	0.30	nd	nd	nd	nd	nd	nd	nd
39.25	2145	Octadecanoic acid	Mild fatty	nd	1.66	nd	nd	nd	nd	nd	nd
40.32	2672	Tetradecanoic acid	Coconut	nd	nd	nd	2.76	nd	nd	nd	nd
40.99	2740	Pentadecanoic acid	Waxy	nd	nd	nd	3.91	nd	nd	nd	nd
42.20	3160	9,12-Octadecadienoic acid	Rancid	0.84	34.64	nd	9.13	nd	nd	nd	nd
43.45	2931	n-Hexadecanoic acid	Waxy	0.64	1.40	1.90	28.85	0.08	nd	nd	nd
44.16	3157	9-Octadecenoic acid	Faint fatty	nd	6.11	nd	2.68	nd	nd	nd	nd
		Alcohols									
17.97	1600	2-Ethyl-1-hexanol	Citrus	nd	nd	nd	nd	nd	0.26	nd	nd
10.14	1396	2-Octanol	Spicy	nd	nd	nd	nd	nd	0.2	nd	nd
12.09	1448	Hexanol	Herbal	nd	nd	nd	nd	1.12	nd	nd	nd
20.26	1621	Linalool	Floral	0.37	nd	0.27	nd	0.41	nd	0.03	nd
23.45	1649	5-Methyl-2-(1-methyl) cyclohexanol	Camphor	nd	nd	nd	nd	nd	0.01	nd	nd
31.62	1778	2-Methoxy-phenol	Smoky	0.10	nd	nd	nd	0.04	nd	nd	nd

Table 7 Continued

RT[A]	RI[B]	Volatile flavour compound	Attributes[C]	Peak areas (%)							
				Liquid-liquid extraction/GC-MS				SPME/GC-MS			
				F	FD	HD	SD	F	FD	HD	SD
		Alcohols									
32.64	1821	2,6-bis(1,1-dimethylethyl)-4-methylphenol	Camphor	0.13	nd	nd	nd	0.04	0.19	nd	nd
32.07	1781	Benzenemethanol	Sweet and fruity	0.53	nd	0.30	nd	0.59	nd	0.24	nd
33.35	1870	Phenylethyl alcohol	Floral odour	nd	0.16	nd	3.95	nd	0.39	nd	nd
34.74	2008	1-Hexadecanol	Waxy floral	nd	nd	nd	2.31	nd	nd	nd	nd
		Ketones									
3.74	1167	1-Penten-3-one	Spicy, pungent	42.93	38.18	37.57	0.90	nd	nd	nd	nd
11.15	1424	3-Hydroxy-2-butanone	Creamy	nd	nd	nd	1.53	0.16	nd	0.17	1.00
23.03	1646	Dihydro-2(3H)-furanone	Creamy	nd	nd	nd	0.63	nd	nd	nd	1.43
25.32	1666	4-Ketoisophorone	Musty, woody, sweet	nd	0.05	nd	nd	nd	nd	nd	nd
32.98	1844	Beta-ionone	Sweet	0.10	0.08	nd	nd	0.11	0.09	nd	nd
35.96	2198	2,3-Dihydro-3,5-dihydroxy-6-methyl-4(4H)-pyranone	Caramel	nd	0.65	nd	0.92	nd	0.71	0.23	0.89
		Furans									
7.90	1330	2-Pentyl-furan	Green	nd	nd	nd	nd	0.42	nd	nd	nd
18.72	1607	2-Acetyl furan	Balsamic	nd	nd	nd	nd	nd	nd	0.22	nd
		Esters									
5.00	1230	Isoamylacetate	Banana-like	1.18	1.33	0.58	nd	2.41	2.52	1.99	nd
9.46	1376	n-Hexyl acetate	Herbal	0.06	nd	nd	nd	0.07	nd	nd	nd

Table 7 Continued

RT[A]	RI[B]	Volatile flavour compound	Attributes[C]	Peak areas (%)							
				Liquid-liquid extraction/GC-MS				SPME/GC-MS			
				F	FD	HD	SD	F	FD	HD	SD
19.95	1618	2-Furanmethanol-acetate	Horseradish	nd	nd	nd	nd	nd	0.04	nd	nd
28.37	1694	2-Hydroxybenzoic acid methyl ester	Wintergreen like	nd	nd	3.18	nd	0.16	0.36	0.1	nd
37.36	1891	Ethyl linoleolate	Faint odour	nd	nd	nd	1.58	nd	nd	nd	nd
40.48	1962	Dibutyl phthalate	Faintly fruity	nd	nd	0.77	1.81	nd	nd	nd	nd
		Pyrroles									
5.48	1248	1-Methyl-1H-pyrrole	Herbal	nd	nd	nd	nd	nd	nd	nd	1.27
33.62	1889	2-Acetylpyrrole	Licorice-like	nd	0.44	0.27	nd	nd	0.47	0.28	nd
		Aldehydes									
2.83	1095	Hexanal	Leafy	nd	nd	nd	nd	0.03	nd	nd	nd
15.46	1536	2-Dococen-1-al	Fatty	nd	nd	nd	nd	0.04	nd	nd	nd
17.26	1583	Furfural	Almond	nd	nd	nd	nd	nd	0.26	3.73	nd
19.25	1612	Benzaldehyde	Almond	nd	nd	nd	nd	nd	nd	0.04	nd
21.41	1631	5-Methylfurfural	Caramel	nd	nd	0.31	nd	nd	nd	0.01	nd
23.43	1649	1, 3-Cyclohexadiene-1-carboxaldehyde	Herbal	nd	nd	0.26	nd	nd	nd	0.13	nd
23.62	1651	Benzeneacetaldehyde	Flora	nd	0.03	nd	nd	nd	nd	0.04	nd
34.11	1937	Hexadecanal	Cardboard	nd	nd	nd	nd	0.51	nd	nd	nd
43.78	1119	3-Chloro-benzaldehyde	Pungent	nd	nd	4.78	nd	nd	nd	nd	nd
		Sulfur containing compounds									
22.76	792	2,3-Butanediol	Onion	nd	nd	nd	1.21	nd	nd	nd	7.40

Table 7 Continued

RT[A]	RI[B]	Volatile flavour compound	Attributes[C]	Peak areas (%)							
				Liquid-liquid extraction/GC-MS				SPME/GC-MS			
				F	FD	HD	SD	F	FD	HD	SD
		Sulfur containing compounds									
12.26	1453	2-Methyl-tridecane	Mild waxy	nd	nd	nd	1.78	nd	nd	nd	1.78
35.85	2180	1,2-Diiodo-ethane	Faint ether-like	1.48	nd	nd	nd	nd	nd	nd	nd
33.38	1872	1,6-Dimethyl-naphthalene	Woody	nd	nd	nd	nd	nd	0.04	nd	nd
		Hydrocarbon compounds									
30.85	1754	1-Methoxy-4-(1-propenyl)-benzene	Aniseed	nd	nd	nd	nd	nd	nd	0.03	nd
39.21	1789	1-Octadecene		0.11	nd	nd	1.70	nd	nd	nd	nd
23.76	1652	4,7,10-Cycloundecatriene	Woody	nd	0.04	nd	nd	nd	nd	nd	nd
4.06	1191	5-Ethyl-undecane	Herbal	nd	nd	nd	nd	nd	0.33	nd	nd
3.61	1157	5-Methyl-undecane	Herbal	nd	nd	nd	0.44	nd	2.6	0.06	0.34
18.55	1605	Alpha-Gurjunene	Woody	nd	nd	0.40	nd	nd	nd	0.41	nd
21.07	1628	Beta-Caryophyllene	Spicy	nd	1.86	nd	nd	nd	2.01	1.13	nd
29.84	1723	Cyclobutylbenzene	Sweet	nd	nd	nd	nd	0.05	nd	nd	nd
4.27	1203	Dodecane	Woody	nd	0.96	nd	nd	nd	0.17	0.92	nd
3.30	1134	Heneicosane	Waxy	nd	0.06	nd	nd	nd	nd	nd	nd
21.73	1634	Hexadecane	Mild waxy	nd	0.2	nd	nd	nd	0.11	nd	nd
17.26	1583	Tetramethylpyrazine	Nutty	nd	nd	nd	nd	nd	nd	nd	3.56
30.35	1839	Trans-anethole	Herbal	0.10	nd	nd	nd	0.11	nd	nd	nd
10.22	1399	Tridecane	Mild waxy	nd	0.71	nd	nd	nd	0.79	0.09	nd
40.28	1100	Undecane	Herbal	nd	nd	2.50	nd	nd	nd	0.21	nd

Note: RT[A] refers to retention time (min); RI[B] refers to retention index that was based on a series of alkane (C8-C40); nd refers to not detected

[C] Reference: http://www. webbook.nist.gov/chemistry/, http://www.flavournet.org/flavournet.html

2. 5 Conclusion

Drying methods influenced on the physical and chemical qualities, hotness and volatile flavour characteristic of Chee fah chilli. The FD sample gave more bright-red colour and contained 1.8 and 3.62 times higher ascorbic acid content than the HD and SD samples. The sun, hot air and freeze drying methods did not affect the capsaicin concentration in all the dried chilli. The extraction techniques, LLE and SPME, were able to differentiate and identify the varieties of volatile flavour compounds from dried chilli samples. The groups of volatile flavour compounds, the acids, ketones, pyrroles, furans and aldehydes, dominated in the dried chilli volatile flavour attributes. These compounds were even possible to identify the volatile flavour characteristic of the dried chilli samples. 1-Penten-3-one was a main pungent odour compound in chilli samples which was only detected by GC-MS. The dominant flavours, such as acetic acid and 2-methylpropionic, were found in SD sample. 2-Acetylpyrrole and furfural were main volatile flavours finding in HD and FD samples. The freeze drying method affected on greater improvement of dried chilli qualities. Nonetheless, the chilli dried by freeze and hot air-drying methods presented good qualities (i.e. moisture content, colour and ascorbic acid) as fit in well in commercial standard range.

CHAPTER 3

SENSORY PROFILE ANALYSIS OF DRIED CHEE FAH CHILLI

3.1 Abstract

The objectives of this research chapter were to investigate (1) sensory characteristics of dried chilli samples produced from different processes and (2) the linkages between objective measurement (on instrumental parameters) and subjective analysis (on sensory profiles). The major volatile (1-penten-3-one; 1P3O) and hotness (capsaicin) compounds in chilli identified by trained panellists were related with instrumental results by Partial Least Squares (PLS) regression and applied to classify the dried chilli samples. A trained panellists (n=15) developed the sensory lexicon consisting of 12 sensory attributes relating to hotness and pungent odour, and the test protocols. The samples were prepared in both ground and solution form. Labelled Magnitude Scale (LMS) was applied for evaluation of the sensory profiles. All dried chilli samples which were prepared in ground form, were found to present lower intensity of hotness than those in solution form. Evaluations of the descriptive sensory profile revealed that freeze chilli (FD) sample presented higher intensity of fresh chilli odour and in most of hotness-related attributes than that of hot air (HD) and sun (SD) dried chilli samples. Interestingly, perceived intensity scores of raise-to-nasal pungent odour, sting-pungent odour and oral sting attributes of FD and HD were not significantly different ($P>0.05$). The darkest red colour and the least hotness and pungent odour were present in SD sample. The highest content of 1P3O was found in FD by GC-MS ($P<0.05$), although the trained panellists could not differentiate the intensity of pungent odour between FD and HD. Whereas hotness intensity of the three dried chilli samples could be differentiated by trained panellists, but no significant difference in capsaicin content was detected ($P>0.05$).

3.2 Introduction

Pungent odour and hotness are sensations which are elicited by stimulation to the free nerve endings of the trigeminal nerve and usually cause

irritations (Reinbach *et al.,* 2007; Frasnelli *et al.,* 2009). The free nerve endings are located in both the nasal or oral cavities (Silver and Maruniak, 1981; Silver *et al*., 2006). The pungent odour and hotness are feature flavour attributes of spices, particularly chilli. Generally, the pungent odour and hotness attributes have been utilised as a condiment of various spicy foods in a form of dried chilli which mostly produced by sun-, hot air- and freeze-drying methods. These attributes contribute with a range of flavours to spicy foods, they also add another dimension to meals which is enjoyed by consumers (Jitbunjerdkul and Kijroongrojana, 2007). The hotness attribute of chilli is imparted by capsaicin (Kobata *et al*., 1998; Bosland, 1996; Walsh and Hoot, 2001). The hotness sensation is described in various terms such as hot, sharply, heat, bite, fiery and a burning sensation by taste reception and mouth burning (Eissa *et al*., 2007; Rodriguez, 2008; Toontom, 2008). Likewise, a sensation perceived by 1-penten-3-one (1P3O) which is a major pungent odour compound of chilli is described as strong odour, sharp pungent odour and pungent sensation through nasal cavity (Luning *et al*., 1995; Van Ruth *et al*., 1995; Tainter and Grenis, 2001).

The particular compounds of chilli (i.e. capsaicin and 1P3O) can be decomposed during drying process (Pordestimo *et al.*, 2004; Luning *et al.*, 1995; Govindarajan, 1986; Venskutonis, 1997; Lin and Durance, 1998; Szumny *et al*., 2010). Decompositions of the compounds influence on different sensorial property perceived by consumers (Pääkkönen *et al.*, 1990) and may not meet the requirement of the consumers in terms of flavour attributes.

Currently, sensory profiles in food products can be delivered by Generic Descriptive Analysis (GDA). The sensory descriptive analysis allows the most suitable philosophies of the various methods to be used. The combination among those methods can be varied according to the needs of the project (Murray, 2001; Delgado and Guinard, 2011). The GDA can be applied to combine different approaches from a variety of methods, particularly the QDA and Sensory Spectrum (Lawless and Heymann, 2010).

GDA would usually be conducted with 8 to 12 panellists. They need to be trained with the use of reference standards to understand and to agree on the meaning of the attributes used. The scale used in GDA is usually a quantitative scale which measures intensity of the attributes and allows the data to be statistically

analysed. This method is conducted in three general stages as following; (1) the first is to train the panellists which can be done by either consensus, ballot or combined training methods, (2) then the panellists performance is to be tested and checked for their consistency and validity, if they are qualified then the third stage is (3) the product evaluation when panellists evaluate the samples at least 2-3 replications by all sensory attributes (Lawless and Heymann, 2010).

The training stage provides panellists 'measurement tools' to be calibrated. With a wide range of products in the specific category, individual panellist will be asked to generate the descriptors and reference standards that needed to describe the differences among the products. Later on, the words needed to be discussed, debated and agreed within the panel in the consensus training. Whereas the ballot training is a 'short cut' method by providing a list of possible descriptors and references that could be used to describe the products. The list is mostly based on existing lexicons and test protocol from previous or routine practice in the same product type. The combined training method is an approach of both consensus and ballot training when the panellists deliver some descriptors on their own through consensus and others are added through suggestions by the panel leader or from the existing lexicons. Once the training section has been completed, the panel performance will be tested for their consistency and validity, as an individual and as a group. If they are qualified, then the samples can be evaluated (Murray *et al*., 2001).

During the first few training sessions, the panellists create a score sheet and they are allowed to decide and adjust on the scale used. Among various scales, an unstructured line scale is most commonly used in sensory descriptive analysis (Lawless and Heymann, 2010). However, a Labelled Magnitude Scale (LMS) is also received interests and has been recently applied in the similar type of sensory work. LMS was originally developed for intensity rating of oral stimuli with the top anchor of the scale representing *the most imaginable* intense oral sensation. The LMS is suggested to use in assessment of intensity of attributes (Cardello *et al*., 2005; Guest *et al*., 2007). This scale was proved to provide high power of data discrimination in assessment of intensity (Diamond and Lawless, 2001; Galindo-Cuspinera *et al*., 2009; Hayes *et al*., 2013). It avoids ceiling effects when the attributes are evaluated while they encounter with other scales, particularly unstructured line scales (Lucchina *et al*.,

1998; Bartoshuk, 2000; Hayes *et al*., 2013). A trained sensory evaluation using LMS is found to deliver a complete profile explaining relationship between odourant concentration and aroma intensity of dimethyltrisulfide, methional, linalool and vanillin standard compounds (Kamadia *et al.,* 2006). The LMS is also reported to be more sensitive measurement scale than others in the classification of bitter taste (Bartoshuk, 2000). In conjunction with the LMS while training the panellists, standardised references can be used to decrease the size of contrast shift effects (Diamond and Lawless, 2001).

The analysis of sensory descriptive data is usually done by means of Analysis of Variance (ANOVA) on the individual attributes. When the sensory descriptive analysis is conducted with many samples and attributes, the comparison of profiles becomes cumbersome either graphically or by means of analysis of variance on all the attributes. In that case, Principal Component Analysis (PCA) can be the most effective tool for an exploration of the data (Vandeginste *et al*., 1998). PCA is a technique based on the transformation of an original data matrix into a smaller set of unrelated or non-orthogonal composites that together account for most of the original matrix's total variance. The objective is to explicate as much of the total variation of the data with as few principal components (PCs) as possible (Allen and Rao, 2000). Each of the components is a linear combination of some of the original variables (Muñoz, 1997) and the orthogonal and non-orthogonal components in relation to the original variables (and samples) are shown in PCA loading factors and PCA bi-plots.

Partial Least Square Regression (PLS) is a technique that generalises and combines aspects from principal component analysis and multiple regressions. It is often used for construction of the models relating sensory data to analytical instruments (Heiniö *et al*., 2003; Zhao *et al*., 2007; Chung *et al*., 2003). It is principally used for the prediction of a set of dependent variables (i.e. responses) from a set of independent variables (i.e., predictors) (Abdi, 2007). PLS is particularly most appropriate analysis method when the number of factors is larger than the number of observations (over fitting) (Tobias, 1997). In such a case, even though the number of factors is large, there may be only a few underlying factors (variables; X) that account for most of the variation in the response (Y). PLS technique extracts these factors, accounting for as much of the manifest factor variation as possible (Zhao *et al*., 2007).

Therefore, PLS should be a suitable statistical tool used to demonstrate the underlying associations between compositional data and sensory attributes (Noble and Ebeler, 2002).

The main objective of this research was to investigate sensory characteristics and establish the sensory profiles of dried chilli samples produced by three different processes, by the trained panel. A secondary objective was to link the data derived from both objective measurement (on instrumental parameters) and subjective analysis (on sensory profiles) by PCA. The associations of instrumental parameters and the key sensory characteristics will be statistically studied by PLS regression method.

3.3 Materials and Methods

3.3.1 Raw materials and Chemicals

Hot air (HD), freeze dried (FD) and sun dried chilli (SD) samples which were produced as mentioned in Chapter 2 were used. Fresh Chee fah chilli (F) (as mentioned in Chapter 2), galangal, ginger, cumin and dried black pepper were purchased from a contact distribution from local market in Songkhla province.

Capsaicin (≥95.0%, from Capsaicum sp) and 1-penten-3-one (1P3O) (97.0%) were purchased from Sigma-Aldrich Co. LLC. (USA). Ethanol (99%, Food grade) was obtained from LabScan Asia Co., Ltd (Bangkok, Thailand).

3.3.2 Sample preparation

The whole pod of F as well as the three dried samples (FD, HD and SD) which were dried until reach a moisture content of 10-13%, packed in aluminium laminated bags under vacuum condition and then stored at -20^{o}C, as mentioned in Chapter 2, were used in this chapter. All chilli samples were prepared in ground and solution form in order to observe whether they could generate different effects on panel perception. Samples in ground form were freshly ground just before use in every session of the entire experiments. Then, the samples were passed to sieve in order to get a typical size of chilli powder (80 meshes), according to Thai Community Product Standard of ground chilli (TCPS 492-2004). On the other hand, samples in solution form, 2.5 g of ground fresh and dried chilli were mixed with 1 l of 2% ethanol. The mixtures were stirred under room temperature for 10 min, filtered by filter paper No.4 and then solution was subjected to the sensory evaluation immediately.

3.3.3 Recruitment and screening panellists for descriptive analysis

Forty five participants were recruited from chilli-users (Mazzone *et al.,* 2007; Reinbach *et al.,* 2009). The recruitment and pre-screening criterion (Ludy and Mattes, 2011) were; 1) age group (18 to 35 years; these ages had high sensory sensitivity as recommend by Cain and Gent (1991) and Shusterman *et al*. (2003)), 2) smoking habit (non-smoker only), 3) frequency of spicy food consumption (at least 1 time/week), 4) willingness to eat capsaicin-contained foods, 5) allergies to materials provided in the study (none), 6) health condition (no chronic illness or regular cold symptoms) and 7) availability on training times (available to attend 2x2 hour sessions per week) (Appendix 4.2, Section 4.2.1). After pre-screening, the candidates were required to perform a screening test for sensory ability. The potential panellists who present high sensitivity were recruited for the training period which was aimed to be completed in 42 training hours.

The 15 participants were recruited to form a panel based on their sensory sensitivity and ability to describe sensations. All of them joined in the training with high scores of correct answers (more than 80%) from the screening questionnaire and ballot test (Meilgaard *et al*., 1999; Cliff and Heymann, 1992; Baron and Penfield, 1996; Cometto-Muñiz *et al*., 2005; Carden *et al*., 1999; Hutchison *et al*., 1990). The screening questionnaire and ballot test are composed of the following four items as shown in Appendix 4.3.

(1) Recognising and identification of aromas related to hotness and pungent samples, i.e. F, SD, HD, FD, 1P3O, galangal and pepper.

(2) Ranking intensities of capsaicin solutions representing 3 sets of hotness levels, i.e. set 1 (0.1, 0.3, 0.4 and 0.8 mg/l), set 2 (1, 2, 4 and 6 mg/l) and set 3 (8, 10, 15 and 20 mg/l), and a set of pungent odour test consists of different concentrations of 1P3O (0.2, 0.4, 0.8 and 1.5 µl/l).

(3) Ability to describe sensations, counted numbers of vocabulary generated in describing the odour and hotness properties of two different chilli samples (i.e. 2.5 g fresh chilli and 2.5 g HD chilli)

(4) Ability to discriminate 1P3O pungent odour and capsaicin hotness samples by 12 sets of 3-AFC test. (Appendix 3.1, Table 1)

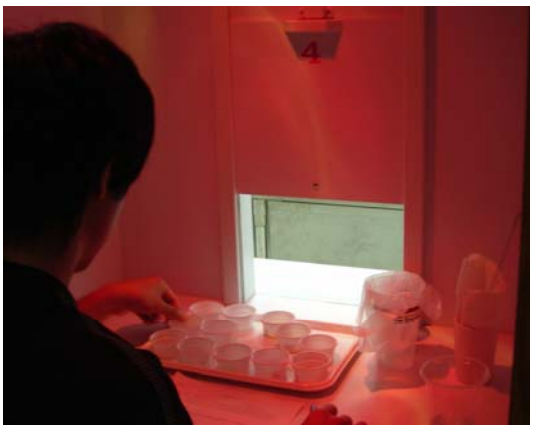


Figure 7 Panel screening test

3.3.4 GDA Training procedures

A generic descriptive analysis (Lawless and Heymann, 2010) was used to develop the language and measurement protocols for the evaluation of all samples. The consensus training method which the all sensory attributes are created by the panel was applied.

The training steps consisted of; (1) Initial orientation session where panellists received detailed explanation about the descriptive sensory methodology and general description of the chilli samples (Appendix 4.4, Section 4.4.1). Samples were selected from range of fresh and dried chilli products in order to span the product space. (2) Lexicon development, it involved with the selection of reference standards and practicing on scaling by rating intensities of selected samples and developed lexicon. This step was aimed to clarify and define the terms, the appropriate reference standards and how to standardise the measurement of each attribute. The final score sheet and test protocol were agreed to accurately measure the test products, then step (3) testing panellist performance and finalise a list of the panel members panel to work on further sample evaluation.

Panellists undertook a 42-hours training programme that included both general and specific training on chilli hotness and pungent odour. All 15 panellists attended the same sessions. Group and individual feedbacks were given to improve the panel performance throughout the training programme.

3.3.4.1 Orientation to the training, exploring the product space and development of lexicon

3.3.4.1.1 Orientation session (hour 1st-2nd): All the potential panellists (n = 15) attended an orientation session. There was a brief presentation on the objectives of the study, the experimental samples (e.g. fresh and dried chilli samples), the perception pathways of hotness and pungent odour, the sensory techniques and the methodology involved in this study. They were informed of the development stages of training and evaluation and agreed on dates, time and location of all the training sessions. During orientation all participants were given an emphasis to the importance of their commitment to the project and their presence during all the group-training sessions.

3.3.4.1.2 General training (hour 3rd-8th): All panellists were given general training. This included group discussions on different types of hotness and pungent odour characteristics presented and perceived in two sets of standard samples (Table 8). (Appendix 3.2, Table 2-4).

Table 8 Concentration of reference samples for both hotness and pungent odour attributes

Samples	**Concentrations (dry weight basis)**	**Volumes per serving used in term development**	
		Hotness attributes (in 3 oz plastic cups)	**Pungent odour attributes (in 250 ml glass jars)**
Standard samples			
Capsaicin standard in 2% ethanol	2.36 mg/l	10 ml	-
1-Penten-3-one standard	0.2- µl/l	-	10 ml
Samples from raw materials			
Ground fresh chilli (F)	2.5 g	2.5 g	2.5 g
Ground sun dried chilli (SD)	2.5 g	2.5 g	2.5 g
Ground hot air dried chilli (HD)	2.5 g	2.5 g	2.5 g
Ground freeze dried chilli (FD)	2.5 g	2.5 g	2.5 g
Ground fresh chilli in 2% ethanol (SF)	2.5 g/l	10 ml	10 ml
Ground sun dried chilli in 2% ethanol (SSD)	2.5 g/l	10 ml	10 ml
Ground hot air dried chilli in 2% ethanol (SHD)	2.5 g/l	10 ml	10 ml
Ground freeze dried chilli in 2% ethanol (SFD)	2.5 g/l	10 ml	10 ml
Ground fresh galangal in 2% ethanol	2.5 g/l	10 ml	10 ml
Ground fresh ginger in 2% ethanol	2.5 g/l	10 ml	10 ml
Ground fresh cumin in 2% ethanol	2.5 g/l	10 ml	10 ml
Ground dried black pepper in 2% ethanol	2.5 g/l	10 ml	10 ml

The panellists were asked to sniff and taste the samples presented in either plastic cups or glass jars. The individual list of perceived sensation terms relating to hotness and pungent odour was summarised, including the perceived intensities of each term and the perceived locations whether in the nose or the mouth or both. Then they were asked to discuss an individual result to come up with a group consensus. At the end of the session, the panel went through the developed lexicon and eliminated redundant terms or those were repetitive or ambiguous. A researcher who was also a panel leader, summarised the list, assisted resolving any confusion, and brought the group to consensus of the final terms.

3.3.4.1.3 Familiarise the panel with LMS scale (hour 9th): Panellists were introduced to six generic verbal descriptors normally used in LMS , namely "barely detectable", "weak", "moderate", "strong", "very strong" and "strongest imaginable" (Green *et al*., 1993). The panellists were instructed to discuss for development of the most appropriate verbal descriptors in Thai based on the generic set and then rank the verbal descriptors according to their perceived degree of quantification. (Appendix 3.2, Table 5).

3.3.3.1.4 Constructing the panel LMS

- Creating LMS by the panellists (hour 10th): The panellists were introduced to 10 cm vertical Labelled Magnitude Scale (LMS) with the six verbal anchors at the following Green *et al*. (1993)'s LMS scale points; "barely detectable" 0.14 cm, "weak" 0.61 cm, "moderate" 1.72 cm, "strong" 3.54 cm, "very strong" 5.33 cm, and "strongest imaginable" 10 cm (Appendix 3.2, Figure 7a). Then, the individual panellist was instructed to freely tune the verbal labels by placing a mark on the LMS using the descriptors previously created in sequence that they felt appropriate and with space between which was not necessary to be in equal interval. The distances of placed descriptors on the scale derived from individual panellists were measured and analysed to obtain the geometric means of the distances. The geometric means are the group means representing the means of each descriptor to be placed on the LMS from every panel members.

The obtained panel LMS also consists of the six verbal descriptors in Thai. Each verbal descriptor was placing a mark on the 10 cm vertical line scale in the Thai LMS, at following points of; barely detectable = 0.13 cm, weak = 0.59 cm,

moderate = 1.64 cm, strong = 3.35 cm, very strong = 5.21, and strongest imaginable = 10 cm (the Thai LMS is shown in Appendix 3.2, Figure 7b). This scale was afterwards compared with another commonly used scale and proved to be the more suitable type. Then it was used in the training sessions onwards and also to evaluate the sensory intensity.

- Establishing the test protocol (hour 11^{th}-14^{th}): During scale training session, the individual panellist also practiced on the scale with reference samples and how to use their 5 senses to measure the sensory attributes. Test protocol includes sensory terms and definition and how the measurement can be done accurately. After that they were established and standardised with the reference samples. (Appendix 3.2, Table 6-7).

- The comparison of LMS and ULS (hour 15^{th}-26^{th}): The panel was offered to use another type of scale – the horizontal 10-cm unstructured line scale (ULS, where 0 is no strength and 10 is strongest imaginable; Appendix 4.4, Section 4.4.1.2) to compare ease of use. Both scales (ULS and LMS) were used by each panellist to measure intensities of the 6 attributes from the same set of samples (1P3O, capsaicin, ground samples and dried chilli solutions) (Appendix 3.2, Table 7-10). The results demonstrated that there was no significant difference of the all intensity scores measured from the two scale types ($P>0.05$) (Table 9). In addition, the panel thought that it was easier to use LMS for the intensity measurement (Appendix 4.4, Section 4.4.1.1). Hence the LMS was selected and agreed within the panel to be used in further training and product profiling.

Table 9 P-value from F-test ANOVA associated with comparisons of intensity scores derived by LMS and ULS

	Significance level (P-value)									
	1P3O	**Capsaicin**	**F**	**FD**	**HD**	**SD**	**SF**	**SFD**	**SHD**	**SSD**
Oral burn (OB)	NA	0.902	0.530	0.559	0.990	0.599	0.846	0.918	0.859	0.573
Oral sting (OS)	NA	0. 836	0.400	0.219	0.202	0.306	0.208	0.927	0.783	0.142
Raise-to-nasal pungent odour (RNO)	0. 954	NA	0.103	0.641	0.277	0.902	0.257	0.735	0.845	0.821
Sting-pungent odour (SPO)	0. 489	NA	0.856	0.802	0.815	0.496	0.813	0.945	0.308	0.852
Warm in mouth (WM)	NA	0.222	0.861	0.908	0.954	0.604	0.890	0.492	0.472	0.432
Tongue numb (TN)	NA	0. 751	0.343	0.720	0.344	0.113	0.184	0.172	0.101	0.759

Note: 1P3O = 1-penten-3-one, F = ground fresh chilli, FD = ground freeze dried chilli, HD = ground hot air dried chilli, SD = ground sun dried chilli, SF = solution of fresh chilli, SFD = solution of freeze dried chilli, SHD = solution of hot air dried chilli and SSD = solution of sun dried chilli
NA refers to not analysed.

3.3.4.2 Panellist performance test

3.3.4.2.1 Reference standard test (hour 27^{th}-32^{nd}): The first performance test was conducted in this session. Each individual panellist assessed 3 standard samples of 1P3O (0.4, 0.8 and 1.5 µl/l 1P3O) and 2 standard samples of capsaicin (2 and 6 mg/l). The five standard samples which covered the range of hotness and pungent odour in the experimental sample set, were presented in triplicates with different sample codes, for the twelve attributes. The performance of individual panellist was determined using Analysis of Variance (ANOVA). Ideally, the insignificance of the effects ‘‘replicates’’, ‘‘samples’’ and ‘‘interaction panellists x samples’’ are preferred in this present study. Because the effects indicate repeatability, discriminate ability and homogeneity of the panel, respectively. In addition, the obtained results will also determine ‘inner scale’ as of whether the individual panellist was consistent in their use of the scales through the triplicates of samples on similar attributes. All panellists appeared to rate each attribute in the same way and they could discriminate between the samples for all 12 attributes, significantly ($P \leq 0.05$). Since not all panellists performed well in this round of the performance check - all panellists were required to continue further training when they would again have their performance tested for reliability and validity before they were qualified and ready to evaluate the sample profiles. (Appendix 3.2, Table 11)

3.3.4.2.2 Panellist training with larger sample set (hour 33rd-36th): All panellists continued training on the new sample set of 3 ground and 3 solution Chee fah chilli samples. The panellists practiced rating samples individually in the partitioned booths with the sample sets. The individuals were spontaneously given the feedback after the each practice, emphasising on whether their own ratings were similar for all triplicates of sample, and whether they scored were in line with the whole panellists. This training session contributed to the development of the individual and synchronizing of the whole panellists from consistency and validity points of view. (Appendix 3.2, Table 12)

3.3.4.2.3 Reliability and validity test on the panel (hour 37th-42nd): The second performance test was conducted to determine the reliability and validity of the panel on all attributes. If the panel performance was consistent and valid on the test samples (known intensities), then they would be ready to start working on the sample evaluation. Each individual panellist assessed 3 samples of dried chilli (0.87, 2.16 and 5.71 g/l HD) in triplicates for the twelve attributes. The panellists (n=15), who could not discriminate between the samples and did not have consistency over the triplicates of samples, were re-trained for two hours. The panellists were made aware of their defective issues and, if no improvements were observed, he/she would not be able to participate in the panel. (Appendix 3.2, Table 13)

During scale training and performance tests, the panellists worked individually to rate samples on LMS scale in the partitioned booths with the sample sets. The sensory evaluation room temperature was controlled at 25°C, and free from distracting noises and odours. In the tests, the panellists followed the assessment test protocol on developed sensory lexicon, the results are shown in Table 11. The flavour-related intensities were assessed on both pungent odour and hotness attributes via mouth only (retronasal perception). While, the pungent odour and hotness –related intensities were assessed via nose and mouth, respectively. During evaluation of hotness-related attributes, the panellists were required to rinse their mouths 1 time with sucrose solution (10% sucrose w/w in water) (Nasrawi and Panborn, 1990), 5 times with water and then wait for 5 min between samples (Lawless *et al*., 2000; Allison *et al.*, 1999). During pungent odour evaluations, the panellists were enquired

to sniff non scent facial tissue paper, then to rest for 1-2 min. and proceed to the next sample (Adapted from Cometto-Muñiz *et al.,* 2000).

Figure 8 Training sessions with chilli samples

3.3.5 Sample evaluation

The sample set consists of the two standard solutions (1P3O and capsaicin), four ground chilli samples (fresh (F) and three dried chilli (FD, HD and SD) and solution of chilli samples (fresh (SF) and three dried chilli (SFD, SHD and SSD)). The ground and diluted dried chilli samples are included in order to observe whether they generate different effects on panel perception.

After training within 1-2 weeks, all the qualified panellists (n =15) evaluated the intensity of hotness and pungent odour of 10 samples in triplicates. The 10 samples were presented in a 30-minute session. Each sample was carried on one replicate per day, 3 days per week for 2 weeks. Balance first-order and carry-over-effect design (MacFie *et al*., 1989) was applied for serving plan. The data set was analysed by a two-way ANOVA with sample, panellist, replication and their interactions.

The pungent odour, hotness and flavour-related intensity were separately evaluated. In pungent odour-related intensity evaluation**,** 10 millilitres of solution sample and 2.5 g of ground sample were served in a glass bottle (Adapted from Cometto-Muñiz *et al.,* 2000). Each sample was presented in an aluminium foil-

covered glass bottle to mask any influences of colour and to control the transferring of any odorant. To evaluate the hotness and flavour intensity, 10 millilitres of solution sample and 2.5 g of ground sample were served in a plastic cup (Adapted from Cometto-Muñiz *et al.,* 2000). Each sample was presented in the booth with red light to mask any colour interference. In case of hotness-related intensity evaluation, the panellists were required to ware nose-clips during the test in order to focus on the perceived intensity in mouth and to block any orthonasal odorants (Adapted from Cliff and Heymann, 1992). In the flavour-related intensity evaluation, however the panellists rated their perceived pungent odour and hotness intensities via mouth without nose-clips.

The sample profiles of 10 samples were monadically evaluated for all 12 attributes, in 6 testing sessions. The evaluation was based on the test protocol including the use of an LMS scale (Appendix 4.4, Section 4.4.2)

3.3.6 Statistical analysis

The sample profiles were evaluated in triplicates. The intensity scores were transformed into geometric means of all panellists' scores and were used in ANOVA for data analysis. The experimental design was a Randomized Complete Block Design (RCBD), as designed for general descriptive analysis test. Significant differences between means were estimated by Duncan's new multiple range test (DMRT), with a level of significance of 0.05. Statistical analyses were performed by using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA, 2002). Principal Component Analysis (PCA) was applied to observe the sensory profiling of the chilli samples which obtained from the panel by XLSTAT software (XLSTAT Pro 2008). Partial Least Squares (PLS) was applied to analyse both data sets derived from the instrumental measurements presented in Chapter 2 (as predicting variables) and from the GDA data (as dependent variables) to reveal the relationships between subjective and objective measurements.

3.4 Results and Discussion

3.4.1 Sensory profiling generated by GDA

The panel of fifteen subjects undertook a 42-hour training program that included general training and training on specific attributes of hotness and pungent odour. The panel generated terms that described hotness and pungent odour attributes as they perceived (Table 10). The initial list of attributes was revised to clarify and remove subjective, duplicate, or ambiguous terms. Upon the discussions, 1) 'sting-pungent' and 'tingle-pungent' odour, 2) 'oral sting' and 'oral biting' sensations were found to be duplicate terms with the same perceptual meaning, after refining the definitions of those terms, only sting-pungent odour and oral sting sensation were remained in the list. Fermented odour was agreed among the panellists that it shared dimensional meaning with fresh chilli odour as opposite word anchors perceived in the research product range. Hence the attributes were then combined into one called fresh chilli odour. The final attribute list consisted of twelve attributes (Table 10). The sensory lexicon which was developed and agreed by all panellists was shown in Table 10 including attribute names, agreed definitions, methods of assessment and reference samples that illustrated dimensional meaning of each attribute.

Table 10 Generation of attribute list

Initial attribute list	**Final attribute list**
Dark red colour	Dark red colour
Fermented odour	Burnt chilli odour
Fresh chilli odour	Fresh chilli odour
Burnt chilli odour	Raise-to-nasal pungent odour
Raise-to-nasal pungent odour	Sting-pungent odour
Sting-pungent odour	Warm in mouth
Tingle-pungent odour	Warm in mouth after spitting
Warm in mouth	Oral burn
Warm in mouth after spitting	Oral burn after spitting
Oral burn	Oral sting
Oral burn after spitting	Oral sting after spitting
Oral sting	Tongue numb
Oral biting	
Oral sting after spitting	
Tongue numb	

Table 11 Sensory lexicon and test protocol

Sensory attributes	Definition	Reference samples		Method of assessment	
		Low intensity	High intensity	Hotness or pungent odour-related intensity	Flavour-related intensity
1. Dark red colour	Degree of dark red colour	0.45 g/l fresh chilli	Tomato sauce (Roza brand)	Assess the dark red colour of sample and look through the samples	Assess the dark red colour of sample, and look through the samples
2. Burnt chilli odour	Degree of burnt odour characteristic which is similar to roast chilli	Pure water	2.5 g ground roast chilli (80^{o}C, 10 min)	Assess the burnt odour of chilli, sniff and hold the breath 3-5 s	Assess the burnt odour of chilli, hold sample in mouth 15 s with minimize movement, expectorate and wait 15 s
3. Fresh chilli odour	Degree of fresh chilli odour which is similar to green odour of fresh chilli	Vinegar	2.5 g ground fresh chilli	Assess the green odour of sample , sniff and hold the breath 3-5 s	Assess the green odour of sample, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s
4. Raise-to-nasal pungent odour	Degree of chilli pungent odour characteristic which irritates upper of nose	Pure water	2.04 µl/l 1P3O	Assess the irritated sensation of the upper nose, sniffing and hold the breath 3-5 s	Assess the irritated sensation of the upper nose, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s
5. Sting-pungent odour	Degree of chilli pungent odour characteristic which induces to nasal sting	Pure water	2.04 µl/l 1P3O	Assess the sting sensation of nose, sniff and hold the breath 3-5 s	Assess the sting sensation of nose, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s
6. Warm in mouth	Degree of warm sensation in mouth during keep a sample in the mouth	Pure water	15 mg/l capsaicin	Assess the warm sensation in mouth with nose-clips, hold sample in mouth 30 s with minimize movement	Assess the warm sensation in mouth, hold sample in mouth 15 s with minimize movement

Table 11 Continued

Sensory attributes	Definition	Reference samples		Method of assessment	
		Low intensity	High intensity	Hotness or pungent odour-related intensity	Flavour-related intensity
7. Warm in mouth after spitting	Degree of warm sensation in mouth after spitting sample	Pure water	15 mg/l capsaicin	Assess the warm sensation in mouth with nose-clips, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s	Assess the warm sensation in mouth, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s
8. Oral burn	Degree of burn sensation in mouth during keep sample in mouth	Pure water	15 mg/l capsaicin	Assess the burn sensation in mouth with nose-clips, hold sample in mouth 15s with minimize movement	Assess the burn sensation in mouth, hold sample in mouth 15 s with minimize movement
9. Oral burn sting after spitting	Degree of burn sensation in mouth after spitting sample	Pure water	15 mg/l Capsaicin	Assess the burn in mouth with nose-clips, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s	Assess the burn sensation in mouth, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s
10. Oral sting	Degree of sting sensation in mouth during keep sample in mouth	Pure water	15 mg/l capsaicin	Assess the sting sensation in mouth with nose-clips, hold sample in mouth 15 s with minimize movement	Assess the burn sensation in mouth, hold sample in mouth 15 s with minimize movement
11. Oral sting after spitting	Degree of sting sensation in mouth after spitting sample	Pure water	15 mg/l capsaicin	Assess the sting sensation in mouth with nose-clips, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s	Assess the sting sensation in mouth, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s
12. Tongue numb	Numbing sensation on tongue	Pure water	15 mg/l capsaicin	Assess the numb sensation on tongue with nose-clips, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s	Assess the numb sensation on tongue, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s

3.4.2 Panellist performance test

In the performance test with 8 samples, the reliability and validity of fifteen panellists were determined on twelve attributes. In the first testing session, 3 standard samples of 1P3O (0.4, 0.8 and 1.5 µl/l 1P3O), 2 standard samples of capsaicin (2 and 6 mg/l capsaicin) were evaluated. In the second session, 3 samples of hot air dried chilli (0.87, 2.23 and 5.71 g/l HD) were assessed by the same panel. Observation of test for normality confirmed that the data were normally distributed for all attributes across the sample sets. Table 12 shows the significance levels associated with normality distribution test of each attribute and sample by Shapiro-wilk test.

Table 12 Significance levels (P-value) associated with normality distribution test

	Significance level (P-value)							
	0.4 µl/l 1P3O	**0.8 µl/l 1P3O**	**1.5 µl/l 1P3O**	**2 mg/l Capsaicin**	**6 mg/l Capsaicin**	**0. 87 g/l HD**	**2.23 g/l HD**	**5.71 g/l HD**
Visual								
Dark red colour (DRC)	NA	NA	NA	NA	NA	0.077	0.090	0.068
Pungent odour-related intensity (orthonasal perception)								
Burnt chilli odour (BO)	NA	NA	NA	NA	NA	0.065	0.057	0.367
Fresh chilli odour (FO)	NA	NA	NA	NA	NA	0.071	0.059	0.222
Raise-to-nasal pungent odour (RNO)	0.079	0.071	0.055	NA	NA	0.094	0.068	0.057
Sting-pungent odour (SPO)	0.064	0.116	0.107	NA	NA	0.112	0.054	0.082
Hotness-related intensity (oral perception with occluded nose)								
Warm in mouth (WM)	NA	NA	NA	0.109	0.200	0.167	0.051	0.057
Warm in mouth after spitting (WMS)	NA	NA	NA	0.109	0.200	0.223	0.051	0.057
Oral burn (OB)	NA	NA	NA	0.057	0.090	0.121	0.067	0.069
Oral burn after spitting (OBS)	NA	NA	NA	0.146	0.114	0.087	0.051	0.153
Oral sting (OS)	NA	NA	NA	0.057	0.090	0.104	0.067	0.069
Oral sting after spitting (OSS)	NA	NA	NA	0.146	0.138	0.087	0.051	0.153
Tongue numb (TN)	NA	NA	NA	0.108	0.096	0.059	0.128	0.110
Flavour-related intensity (retronasal perception)								
Burnt chilli odour (BO)	NA	NA	NA	NA	NA	0.116	0.160	0.061
Fresh chilli odour (FO)	NA	NA	NA	NA	NA	0.066	0.132	0.082
Raise-to-nasal pungent odour (RNO)	0.100	0.098	0.058	NA	NA	0.070	0.053	0.376
Sting-pungent odour (SPO)	0.149	0.101	0.118	NA	NA	0.170	0.406	0.647
Warm in mouth (WM)	NA	NA	NA	0.063	0.060	0.067	0.145	0.069
Warm in mouth after spitting (WMS)	NA	NA	NA	0.088	0.334	0.063	0.067	0.135
Oral burn (OB)	NA	NA	NA	0.078	0.066	0.059	0.102	0.053
Oral burn after spitting (OBS)	NA	NA	NA	0.064	0.086	0.174	0.211	0.406
Oral sting (OS)	NA	NA	NA	0.065	0.069	0.099	0.069	0.145
Oral sting after spitting (OSS)	NA	NA	NA	0.067	0.059	0.069	0.072	0.458
Tongue numb (TN)	NA	NA	NA	0.064	0.119	0.102	0.087	0.077

Note: NA refers to not analysed. 1P3O = 1-penten-3-one, HD = ground hot air dried chilli

Table 13 shows a summary of the level of significance associated with the factors and interaction terms in the ANOVA for each of the attributes. The ANOVA shows that there was no significant interaction between panellists and sample, neither with panellist and replication (P>0.05). The reliability of the panel was presented by not having significant effects of replication, panellist, sample-replication interaction and panellist-replication effects (P>0.05). There was a significant sample effect for all attributes which indicated that the panel was able to discriminate the test samples. The results of the sample *post hoc* Duncan's multiple-range test and mean scores for each sample are shown in Table 14.

Table 13 Significance levels (P-value) associated with ANOVA on panellist performance testing

	Significance level (P-value)					
	Sample	**Panellist**	**Replication**	**Sample x Replication**	**Panellist x Replication**	**Sample x Panellist**
Visual						
Dark red colour (DRC)	0.000	0.604	0.411	0.727	0.213	0.806
Pungent odour-related intensity (orthonasal perception)						
Burnt chilli odour (BO)	0.000	0.606	0.395	0.729	0.216	0.802
Fresh chilli odour (FO)	0.000	0.998	0.678	0.793	0.94	1.000
Raise-to-nasal pungent odour (RNO)	0.000	0.643	0.935	0.271	0.376	0.976
Sting-pungent odour (SPO)	0.000	0.740	0.643	0.104	0.277	0.792
Hotness-related intensity (oral perception with nose-clip)						
Warm in mouth (WM)	0.000	0.737	0.527	0.124	0.488	0.456
Warm in mouth after spitting (WMS)	0.000	0.818	0.609	0.086	0.463	0.609
Oral burn (OB)	0.000	0.075	0.636	0.356	0.887	0.234
Oral burn after spitting (OBS)	0.000	0.356	0.346	0.566	0.874	1.000
Oral sting (OS)	0.000	0.250	0.302	0.183	0.187	0.866
Oral sting after spitting (OSS)	0.000	0.314	0.201	0.475	0.263	**0.000**
Tongue numb (TN)	0.000	0.314	0.302	0.230	0.300	**0.001**
Flavour-related intensity (retronasal perception)						
Burnt chilli odour (BO)	0.000	0.976	0.795	0.819	0.095	1.000
Fresh chilli odour (FO)	0.000	0.584	0.833	0.123	0.429	0.952
Raise-to-nasal pungent odour (RNO)	0.000	0.626	0.655	0.101	0.368	0.678
Sting-pungent odour (SPO)	0.000	0.533	0.272	0.069	0.580	0.357
Warm in mouth (WM)	0.000	0.803	0.653	0.082	0.831	0.496
Warm in mouth after spitting (WMS)	0.000	0.060	0.581	0.276	0.904	0.215
Oral burn (OB)	0.000	0.214	0.466	0.570	0.824	1.000
Oral burn after spitting (OBS)	0.000	0.251	0.327	0.221	0.157	0.665
Oral sting (OS)	0.000	0.844	0.115	0.399	0.220	0.495
Oral sting after spitting (OSS)	0.000	0.319	0.344	0.300	0.357	**0.000**
Tongue numb (TN)	0.000	0.314	0.466	0.570	0.824	1.000

The ANOVA results indicate that there was significant interaction between panellist and sample on 2 attributes ($P \leq 0.05$); tongue numb (TN) in flavour-related intensity assessment (retronasal perception) and oral sting after spitting (OSS) in both hotness and flavour-related intensity assessment. The interaction plots in Figure 9 explains the interaction effects as the scores from panellists ID12 and ID 15 were different from other panellists in terms of OSS magnitude (Figure 9a and 9b) This could be an evidence of inconsistency in panellist evaluation process. Whereas panellist ID9 disagreed with the panel on the rank order of TN attribute (Figure 9c). These performances indicate that the three panellists disagreed with the rest of the panel on the attributes across the samples.

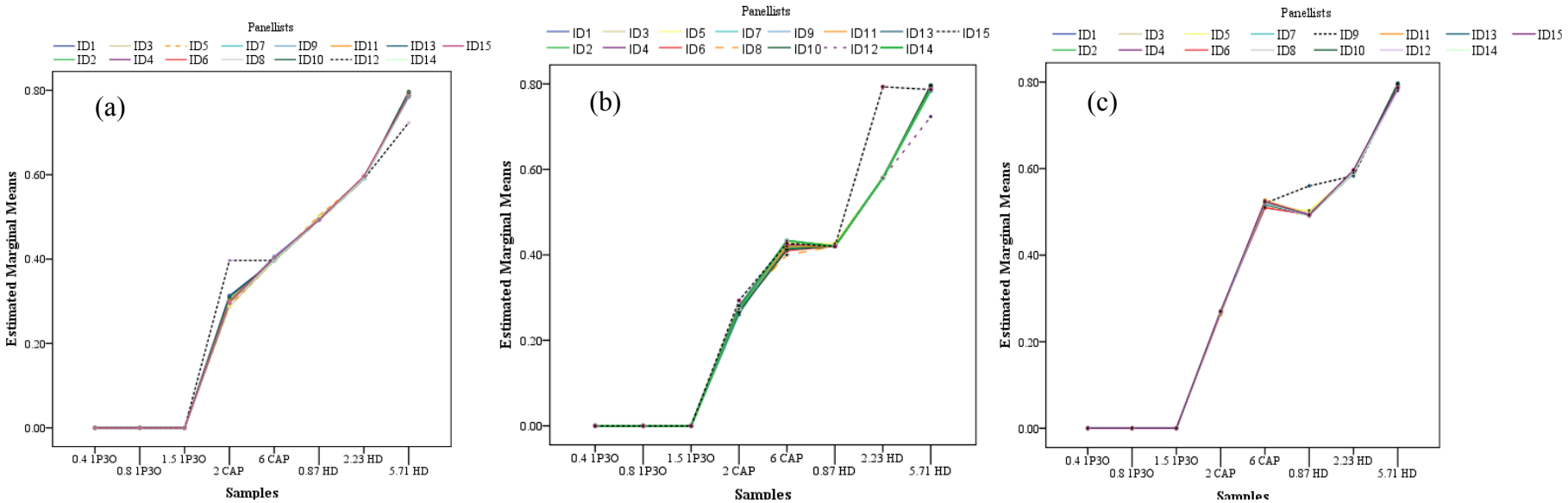


Figure 9 Sample-panellists interaction plot in the perception of OSS in flavour-related intensity (a), OSS in hotness-related intensity (b) and TN (c) hotness-related intensity

Table 14 Mean scores on sample attributes from panellist performance tests

	Samples							
	1P3O			Capsaicin		HD		
	0.4 µl/l	**0.8 µl/l**	**1.5 µl/l**	**2 mg/l**	**6 mg/l**	**0.87 g/l**	**2.23 g/l**	**5.71 g/l**
Visual								
Dark red colour (DRC)	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.07 ± 0.01^{c}	0.48 ± 0.03^{b}	0.85 ± 0.03^{a}
Pungent odour-related intensity (orthonasal perception)								
Burnt chilli odour (BO)	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.28 ± 0.07^{c}	0.49 ± 0.04^{b}	0.55 ± 0.06^{a}
Fresh chilli odour (FO)	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.40 ± 0.04^{c}	0.49 ± 0.03^{b}	0.62 ± 0.04^{a}
Raise-to-nasal pungent odour (RNO)	0.13 ± 0.02^{f}	0.39 ± 0.05^{e}	0.56 ± 0.04^{d}	0.00^{g}	0.00^{g}	0.57 ± 0.04^{c}	0.63 ± 0.03^{b}	0.71 ± 0.04^{a}
Sting-pungent odour (SPO)	0.18 ± 0.03^{f}	0.33 ± 0.08^{e}	0.41 ± 0.06^{d}	0.00^{g}	0.00^{g}	0.39 ± 0.04^{c}	0.47 ± 0.08^{b}	0.56 ± 0.05^{a}
Hotness-related intensity (oral perception with nose-clip)								
Warm in mouth (WM)	0.00^{f}	0.00^{f}	0.00^{f}	0.26 ± 0.06^{e}	0.40 ± 0.06^{d}	0.42 ± 0.03^{c}	0.44 ± 0.07^{b}	0.46 ± 0.07^{a}
Warm in mouth after spitting (WMS)	0.00^{f}	0.00^{f}	0.00^{f}	0.27 ± 0.05^{e}	0.50 ± 0.06^{d}	0.52 ± 0.03^{c}	0.54 ± 0.08^{b}	0.58 ± 0.07^{a}
Oral burn (OB)	0.00^{f}	0.00^{f}	0.00^{f}	0.24 ± 0.07^{e}	0.38 ± 0.07^{d}	0.41 ± 0.06^{c}	0.50 ± 0.06^{b}	0.59 ± 0.05^{a}
Oral burn after spitting (OBS)	0.00^{f}	0.00^{f}	0.00^{f}	0.27 ± 0.01^{e}	0.46 ± 0.07^{d}	0.33 ± 0.03^{c}	0.59 ± 0.05^{b}	0.76 ± 0.05^{a}
Oral sting (OS)	0.00^{f}	0.00^{f}	0.00^{f}	0.25 ± 0.09^{e}	0.43 ± 0.06^{d}	0.44 ± 0.07^{c}	0.50 ± 0.06^{b}	0.60 ± 0.05^{a}
Oral sting after spitting (OSS)	0.00^{f}	0.00^{f}	0.00^{f}	0.28 ± 0.03^{e}	0.49 ± 0.06^{d}	0.35 ± 0.03^{c}	0.61 ± 0.05^{b}	0.78 ± 0.05^{a}
Tongue numb (TN)	0.00^{f}	0.00^{f}	0.00^{f}	0.29 ± 0.05^{e}	0.42 ± 0.02^{d}	0.47 ± 0.05^{c}	0.57 ± 0.04^{b}	0.67 ± 0.04^{a}
Flavour-related intensity (retronasal perception)								
Burnt chilli odour (BO)	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{f}	0.40 ± 0.01^{c}	0.50 ± 0.02^{b}	0.59 ± 0.01^{a}
Fresh chilli odour (FO)	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{f}	0.50 ± 0.01^{c}	0.52 ± 0.03^{b}	0.60 ± 0.04^{a}
Raise-to-nasal pungent odour (RNO)	0.16 ± 0.01^{f}	0.27 ± 0.01^{e}	0.39 ± 0.01^{d}	0.00^{g}	0.00^{g}	0.46 ± 0.02^{c}	0.57 ± 0.01^{b}	0.79 ± 0.03^{a}
Sting-pungent odour (SPO)	0.23 ± 0.01^{f}	0.46 ± 0.02^{e}	0.52 ± 0.02^{d}	0.00^{g}	0.00^{g}	0.45 ± 0.01^{c}	0.53 ± 0.01^{b}	0.59 ± 0.01^{a}
Warm in mouth (WM)	0.00^{f}	0.00^{f}	0.00^{f}	0.23 ± 0.01^{e}	0.40 ± 0.01^{c}	0.46 ± 0.02^{d}	0.57 ± 0.01^{b}	0.79 ± 0.03^{a}

Table 14 Continued

	Samples							
	1P3O			Capsaicin		HD		
	0.4 µl/l	**0.8 µl/l**	**1.5 µl/l**	**2 mg/l**	**6 mg/l**	**0.87 g/l**	**2.23 g/l**	**5.71 g/l**
Warm in mouth after spitting (WMS)	0.00^{f}	0.00^{f}	0.00^{f}	0.26 ± 0.03^{e}	0.36 ± 0.01^{d}	0.38 ± 0.02^{c}	0.51 ± 0.01^{b}	0.69 ± 0.03^{a}
Oral burn (OB)	0.00^{f}	0.00^{f}	0.00^{f}	0.27 ± 0.01^{e}	0.44 ± 0.04^{d}	0.52 ± 0.03^{c}	0.64 ± 0.03^{b}	0.81 ± 0.03^{a}
Oral burn after spitting (OBS)	0.00^{f}	0.00^{f}	0.00^{f}	0.27 ± 0.02^{e}	0.37 ± 0.02^{d}	0.40 ± 0.01^{c}	0.65 ± 0.01^{b}	0.82 ± 0.04^{a}
Oral sting (OS)	0.00^{f}	0.00^{f}	0.00^{f}	0.28 ± 0.02^{e}	0.42 ± 0.01^{d}	0.42 ± 0.01^{c}	0.61 ± 0.07^{b}	0.78 ± 0.07^{a}
Oral sting after spitting (OSS)	0.00^{f}	0.00^{f}	0.00^{f}	0.31 ± 0.03^{e}	0.40 ± 0.01^{d}	0.49 ± 0.01^{c}	0.59 ± 0.02^{b}	0.80 ± 0.08^{a}
Tongue numb (TN)	0.00^{f}	0.00^{f}	0.00^{f}	0.27 ± 0.01^{e}	0.44 ± 0.01^{d}	0.52 ± 0.01^{c}	0.64 ± 0.01^{b}	0.83 ± 0.03^{a}

Note: Different superscripts in a column refer to the significant difference ($P\leq0.05$).

1P3O = 1-penten-3-one, HD = ground hot air dried chilli

The analysis of the interaction plots for TN in hotness-related intensity, OSS in flavour and hotness-related intensity assessment reveals that the interactions were affected by the three panellists (IDs 9, 12 and 15). This means that the way of their hotness intensity interpretation and/ or perception were different from the rest of the panellist. The individual panellist was allowed to have different scoring patterns if he/she was consistent (reliable) across all samples and attributes. However, this was not considered to be a large variation as a range differences between 5.00 and 13.89 % of the means which was the variation of one or two samples (Table 15). Standard deviations (SD) of the three panellists' rating were small and similar to global panel SD for each sample and attributes. This shows that the panellists had individually consistent of the rating on the same sample in the replicates. However, these panellists were given feedback to enable them to adjust their ratings in harmony with the group.

Table 15 Duncan *post hoc* test on oral sting after spitting (OSS) in flavour and hotness intensity and tongue numb (TN) in hotness intensity assessment

Panellists	OSS* of 2 mg/l Capsaicin	TN** of 0.87 g/l HD	OSS** of 2.23 g/l HD	OSS** of 5.71 g/l HD	OSS** of 5.71 g/l HD
ID1	0.31±0.04 ^{b}	0.46±0.02 ^{bc}	0.58±0.04 ^{c}	0.79±0.04 ^{a}	0.80±0.08 ^{a}
ID2	0.30±0.02 ^{b}	0.46±0.05 ^{bc}	0.61±0.05 ^{bc}	0.78±0.03 ^{a}	0.79±0.07 ^{a}
ID3	0.29±0.03 ^{b}	0.46±0.03 ^{bc}	0.59±0.03 ^{bc}	0.77±0.04 ^{a}	0.80±0.09 ^{a}
ID4	0.30±0.02 ^{b}	0.47±0.06 ^{b}	0.58±0.05 ^{c}	0.79±0.05 ^{a}	0.81±0.08 ^{a}
ID5	0.29±0.03 ^{b}	0.47±0.05 ^{b}	0.61±0.06 ^{bc}	0.78±0.05 ^{a}	0.82±0.07 ^{a}
ID6	0.30±0.02 ^{b}	0.46±0.06 ^{bc}	0.58±0.04 ^{c}	0.79±0.06 ^{a}	0.79±0.08 ^{a}
ID7	0.30±0.02 ^{b}	0.47±0.04 ^{b}	0.59±0.05 ^{bc}	0.78±0.04 ^{a}	0.81±0.09 ^{a}
ID8	0.30±0.02 ^{b}	0.48±0.05 ^{b}	0.57±0.06 ^{c}	0.79±0.04 ^{a}	0.79±0.06 ^{a}
ID9	0.30±0.02 ^{b}	**0.56±0.04** ^{a}	0.58±0.05 ^{c}	0.78±0.06 ^{a}	0.79±0.08 ^{a}
ID10	0.31±0.02 ^{b}	0.46±0.06 ^{bc}	0.60±0.04 ^{bc}	0.80±0.06 ^{a}	0.80±0.08 ^{a}
ID11	0.30±0.03 ^{b}	0.47±0.06 ^{b}	0.61±0.06 ^{bc}	0.79±0.05 ^{a}	0.79±0.05 ^{a}
ID12	**0.40±0.02** ^{a}	0.48±0.05 ^{b}	0.59±0.04 ^{bc}	**0.72±0.10** ^{b}	**0.74±0.07** ^{b}
ID13	0.30±0.02 ^{b}	0.46±0.04 ^{bc}	0.58±0.03 ^{c}	0.79±0.05 ^{a}	0.81±0.08 ^{a}
ID14	0.31±0.03 ^{b}	0.47±0.03 ^{b}	0.59±0.03 ^{bc}	0.78±0.06 ^{a}	0.79±0.09 ^{a}
ID15	0.29±0.04 ^{b}	0.46±0.05 ^{c}	**0.80±0.04** ^{a}	**0.80±0.05** ^{a}	0.80±0.09 ^{a}

Note: Different superscripts in a column refer to the significant difference (P≤0.05).
ID1-ID 15 refers to panellists. 1P3O = 1-penten-3-one, HD = ground hot air dried chilli
* refers to retronasal perception.
** refers to oral perception with nose-clip.

3.4.3 Sample evaluation

After training, 15 panellists evaluated 10 samples on 12 attributes in triplicates. The results from the two-way ANOVA were shown in Table 16. The sample effects were significance across all attributes. Whereas, there were no significant interactions between sample and replication, panellist and replication, or sample and panellists. This indicates that the panellists were able to discriminate the samples for all attributes and they were in agreement with the panellist group. The attribute group means for each sample were presented in Table 17. The significant differences of samples do exist according to ANOVA (Table 16) and *post hoc* Duncan tests. The fresh and dried chilli samples were differentiated by all attributes.

Table 16 Significance level (P-value) associated with ANOVA by 12 attributes of experimental samples

	Significance level (P-value)					
	Sample	**Panellist**	**Replication**	**Sample x Replication**	**Panellist x Replication**	**Sample x Panellist**
Visual						
Dark red colour (DRC)	0.000	0.387	0.749	0.167	0.232	0.850
Pungent odour-related intensity (orthonasal perception)						
Burnt chilli odour (BO)	0.000	0.770	0.641	0.084	0.228	0.284
Fresh chilli odour (FO)	0.000	0.614	0.197	0.127	0.160	1.000
Raise-to-nasal pungent odour (RNO)	0.000	0.108	0.628	0.342	0.788	0.977
Sting-pungent odour (SPO)	0.000	0.547	0.488	0.638	0.124	0.992
Hotness-related intensity (oral perception with nose-clip)						
Warm in mouth (WM)	0.000	0.569	0.311	0.102	0.691	1.000
Warm in mouth after spitting (WMS)	0.000	0.448	0.106	0.237	0.499	0.992
Oral burn (OB)	0.000	0.231	0.532	0.194	0.783	0.987
Oral burn after spitting (OBS)	0.000	0.935	0.529	0.578	0.138	0.999
Oral sting (OS)	0.000	0.599	0.333	0.707	0.087	0.951
Oral sting after spitting (OSS)	0.000	0.417	0.231	0.429	0.349	0.995
Tongue numb (TN)	0.000	0.322	0.345	0.728	0.708	0.999
Flavour-related intensity (retronasal perception)						
Burnt chilli odour (BO)	0.000	0.517	0.149	0.075	0.459	0.818
Fresh chilli odour (FO)	0.000	0.268	0.953	0.054	0.214	0.887
Raise-to-nasal pungent odour (RNO)	0.000	0.137	0.549	0.073	0.545	0.666
Sting-pungent odour (SPO)	0.000	0.911	0.485	0.793	0.078	0.997
Flavour-related intensity (retronasal perception)						
Warm in mouth (WM)	0.000	0.789	0.158	0.284	0.225	0.957
Warm in mouth after spitting (WMS)	0.000	0.539	0.746	0.159	0.370	0.874
Oral burn (OB)	0.000	0.202	0.234	0.343	0.882	0.950
Oral burn after spitting (OBS)	0.000	0.789	0.158	0.284	0.225	0.957
Oral sting (OS)	0.000	0.351	0.952	0.077	0.265	0.958
Oral sting after spitting (OSS)	0.000	0.469	0.819	0.333	0.285	0.968
Tongue numb (TN)	0.000	0.281	0.962	0.580	0.083	0.923

Table 17 Mean scores of 12 attributes of experimental samples

	Samples									
	F	**FD**	**HD**	**SD**	**SF**	**SFD**	**SHD**	**SSD**	**1P3O**	**Capsaicin**
Visual										
Dark red colour (DRC)	0.81±0.02^{c}	0.72±0.04^{d}	0.85±0.04^{b}	0.90±0.02^{a}	0.02±0.14^{h}	0.26±0.08^{g}	0.47±0.04^{f}	0.54±0.05^{e}	0^{i}	0^{i}
Pungent odour intensity (orthonasal perception)										
Burnt chilli odour (BO)	0^{g}	0.56±0.03^{b}	0.81±0.03^{a}	0.25±0.01^{d}	0^{f}	0.18±0.09^{e}	0.34±0.05^{c}	0.06±0.00^{f}	0^{g}	0^{g}
Fresh chilli odour (FO)	0.79±0.02^{a}	0.26±0.01^{d}	0.22±0.01^{e}	0.04±0.01^{g}	0.37±0.07^{b}	0.35±0.04^{bc}	0.34±0.04^{c}	0.15±0.06^{f}	0^{h}	0^{h}
Raise-to-nasal pungent odour (RNO)	0.53±0.02^{a}	0.24±0.08^{bc}	0.23±0.04^{c}	0.19±0.04^{d}	0.25±0.09^{b}	0.16±0.08^{ef}	0.12±0.07^{f}	0.11±0.08^{f}	0.09±0.09^{g}	0^{h}
Sting-pungent odour (SPO)	0.64±0.02^{a}	0.59±0.05^{b}	0.60±0.07^{b}	0.45±0.01^{cd}	0.47±0.01^{c}	0.45±0.01^{cd}	0.43±0.09^{d}	0.39±0.09^{e}	0.11±0.09^{f}	0^{g}
Hotness-related intensity (oral perception with nose-clip)										
Warm in mouth (WM)	0.09±0.04^{g}	0.39±0.08^{c}	0.36±0.109^{d}	0.10±0.03^{f}	0.36±0.08^{d}	0.49±0.07^{a}	0.41±0.08^{b}	0.39±0.09^{c}	0^{h}	0.27±0.07^{e}
Warm in mouth after spitting (WMS)	0.05±0.02^{g}	0.42±0.08^{c}	0.38±0.01^{d}	0.17±0.05^{f}	0.43±0.06^{c}	0.52±0.02^{a}	0.47±0.01^{b}	0.45±0.08^{b}	0^{h}	0.30±0.06^{e}
Oral burn (OB)	0.15±0.01^{i}	0.49±0.03^{e}	0.45±0.06^{f}	0.37±0.05^{g}	0.54±0.04^{d}	0.65±0.02^{a}	0.63±0.02^{b}	0.58±0.04^{c}	0^{j}	0.29±0.05^{h}
Oral burn after spitting (OBS)	0.22±0.06^{g}	0.50±0.05^{d}	0.48±0.08^{e}	0.48±0.06^{e}	0.55±0.06^{c}	0.65±0.03^{a}	0.66±0.03^{a}	0.58±0.04^{b}	0^{h}	0.32±0.09^{f}
Oral sting (OS)	0.21±0.08^{f}	0.39±0.05^{e}	0.39±0.05^{e}	0.18±0.04^{f}	0.48±0.03^{d}	0.64±0.03^{a}	0.54±0.03^{b}	0.52±0.02^{c}	0^{g}	0.39±0.06^{e}
Oral sting after spitting (OSS)	0.24±0.06^{e}	0.42±0.06^{cd}	0.40±0.03^{d}	0.25±0.08^{e}	0.51±0.02^{b}	0.65±0.03^{a}	0.56±0.02^{b}	0.54±0.04^{b}	0^{f}	0.42±0.02^{c}
Tongue numb (TN)	0.20±0.09^{g}	0.47±0.04^{e}	0.39±0.04^{f}	0.38±0.05^{f}	0.54±0.03^{d}	0.65±0.03^{a}	0.63±0.02^{b}	0.56±0.04^{c}	0^{h}	0.47±0.05^{e}

Table 17 Continued

	Samples									
	F	**FD**	**HD**	**SD**	**SF**	**SFD**	**SHD**	**SSD**	**1P3O**	**Capsaicin**
Flavour-related intensity (retronasal perception)										
Burnt chilli odour (BO)	0^{g}	0.61 ± 0.07^{b}	0.91 ± 0.02^{a}	0.55 ± 0.04^{c}	0^{h}	0.30 ± 0.04^{e}	0.35 ± 0.09^{d}	0.18 ± 0.02^{f}	0^{g}	0^{g}
Fresh chilli odour (FO)	0.92 ± 0.02^{a}	0.44 ± 0.07^{d}	0.39 ± 0.08^{e}	0.25 ± 0.12^{g}	0.63 ± 0.07^{b}	0.58 ± 0.04^{b}	0.49 ± 0.03^{c}	0.28 ± 0.13^{f}	0^{h}	0^{h}
Raise-to-nasal pungent odour (RNO)	0.72 ± 0.05^{a}	0.64 ± 0.06^{b}	0.64 ± 0.07^{b}	0.59 ± 0.05^{c}	0.56 ± 0.05^{d}	0.55 ± 0.06^{d}	0.49 ± 0.05^{e}	0.43 ± 0.08^{f}	0.33 ± 0.07^{g}	0^{h}
Sting-pungent odour (SPO)	0.55 ± 0.07^{a}	0.49 ± 0.10^{b}	0.48 ± 0.07^{b}	0.40 ± 0.08^{cd}	0.39 ± 0.06^{cd}	0.38 ± 0.07^{d}	0.34 ± 0.10^{e}	0.32 ± 0.09^{e}	0.15 ± 0.09^{f}	0^{g}
Warm in mouth (WM)	0.22 ± 0.09^{e}	0.46 ± 0.08^{b}	0.42 ± 0.09^{c}	0.36 ± 0.06^{d}	0.40 ± 0.08^{c}	0.52 ± 0.05^{a}	0.47 ± 0.08^{b}	0.43 ± 0.08^{c}	0^{f}	0.42 ± 0.08^{c}
Warm in mouth after spitting (WMS)	0.41 ± 0.01^{e}	0.60 ± 0.06^{c}	0.53 ± 0.05^{d}	0.41 ± 0.09^{e}	0.60 ± 0.07^{c}	0.68 ± 0.04^{a}	0.66 ± 0.04^{b}	0.65 ± 0.05^{b}	0^{f}	0.39 ± 0.07^{e}
Oral burn (OB)	0.26 ± 0.01^{g}	0.51 ± 0.07^{d}	0.45 ± 0.08^{e}	0.41 ± 0.07^{f}	0.58 ± 0.07^{c}	0.67 ± 0.06^{a}	0.64 ± 0.06^{b}	0.59 ± 0.06^{c}	0^{i}	0.32 ± 0.06^{g}
Oral burn after spitting (OBS)	0.28 ± 0.013^{g}	0.64 ± 0.05^{b}	0.61 ± 0.05^{c}	0.47 ± 0.06^{e}	0.52 ± 0.07^{d}	0.83 ± 0.04^{a}	0.82 ± 0.04^{a}	0.82 ± 0.05^{a}	0^{h}	0.43 ± 0.06^{f}
Oral sting (OS)	0.23 ± 0.01^{h}	0.44 ± 0.08^{e}	0.41 ± 0.05^{f}	0.35 ± 0.04^{g}	0.49 ± 0.07^{d}	0.67 ± 0.05^{a}	0.58 ± 0.06^{b}	0.54 ± 0.06^{c}	0^{i}	0.41 ± 0.07^{ef}
Oral sting after spitting (OSS)	0.24 ± 0.01^{h}	0.47 ± 0.06^{e}	0.43 ± 0.06^{f}	0.26 ± 0.06^{g}	0.59 ± 0.06^{d}	0.79 ± 0.04^{a}	0.77 ± 0.05^{b}	0.74 ± 0.07^{c}	0^{f}	0.47 ± 0.06^{d}
Tongue numb (TN)	0.24 ± 0.03^{e}	0.48 ± 0.06^{c}	0.41 ± 0.12^{d}	0.40 ± 0.09^{d}	0.57 ± 0.07^{b}	0.67 ± 0.06^{a}	0.66 ± 0.04^{a}	0.58 ± 0.07^{b}	0^{f}	0.49 ± 0.06^{c}

Note: Different superscripts in a row refer to the significant difference ($P\leq0.05$).
1P3O = 1-penten-3-one, F = ground fresh chilli, FD = ground freeze dried chilli, HD = ground hot air dried chilli, SD = ground sun dried chilli, SF = solution of fresh chilli, SFD = solution of freeze dried chilli, SHD = solution of hot air dried chilli, SSD = solution of sun dried chilli

3.4.3.1 Colour attribute

Both ground and solution of sun dried chilli samples (SD and SSD) were more darken-red colour than other dried chilli samples ($P \leq 0.05$) in both sample forms (ground and solution). Sun dried chilli sample had less light (L*) and redness (a*) values (L*=32.95±0.68, a*= 10.83±0.55 and b*= 4.61±0.21) than other dried chilli (Data from Chapter 2). This can be explained that sun dried chilli sample exposed more to the air and also took a longer time to dry (Mangaraj *et al.*, 2001; Daood *et al.*, 1996). Topuz and Ozdemir (2004) confirmed that this sun dried chilli was undergone higher colour degradation because of the reaction of pigment oxidation and decomposition. This is perhaps due to the higher exposure to oxygen when an intensive vaporization takes place on the surface of the chilli.

Hot dried chilli samples presented more darken-red colour than the sun dried chilli samples. Since, the hot dried chilli samples used the higher temperature than sun dried chilli. Lee *et al*. (1991) suggested that this mechanism of colour changes is also related to the concentrations of reducing sugar and amino acid of in the pericarp of chilli, which can produce the non-enzymatic browning. Whereas, the freeze-dried chilli samples were produced by low temperature during the process, thus the minimal colour change had occurred. Caparino *et al*. (2012) and Krokida and Maroulis (2001) supported that the poor internal heat transfer during freeze drying process help to prevent browning reaction of the product.

3.4.3.2 The perceived pungent odour and hotness related-attributes

3.4.3.2.1 Retronasal and Orthonasal perceptions: The intensities of pungent odour related-attributes perceived via mouth without nose-clip (flavour-related intensity) – in other word, retronasal perception, tended to be higher than the same attributes when perceived via nasal (orthonasal perception). Generally, odours can reach the olfactory receptor neurons (ORNs) by two routes. One is orthonasally, when volatiles enter the nasal cavity during inhalation or sniffing. Another is retronasally, when food volatiles released in the mouth pass into the nasal cavity during exhalation or eating (Rozin, 1982). The information delivered by each route may differ in its cognitive impact. Humans are more sensitive to retronasal stimulation by sipping or chewing than orthonasal stimulation by sniffing (Voirol and

Daget, 1986). In addition, the congruency of taste and aroma stimulus might increase odour intensity of the sample, when a sample was held in mouth (Schifferstein and Verlegh, 1996; Prescott, 1999; Delwiche and Heffelfinger, 2005).

Likewise, the perception via nose-clip seemed to obstruct the detection of hotness-related attributes. The hotness related-attributes perceived via mouth without nose-clip (flavour-related intensity) tended to be higher intensities than the same attributes perceived via mouth with nose-clip. This was agreed with previous researches (Zacarias *et al*., 2001; Lawlees *et al*., 2004; Mojet *et al*., 2004) which were reported that the panellist who did not wear a nose-clip took more advantage of their retronasal olfactory for taste perception (Mojet *et al.*, 2003). Lim *et al.* (2008) supported that using nose-clips obstructed retronasal olfactory stimulation during tasting and then only oral was perceived.

3.4.3.2.2 Attribute perception in different form of sample: The panellists could not detect the intensity of hotness related-attributes of 0.2 µl/l 1P3O and pungent odour related-attributes of 2.36 mg/l capsaicin samples. Whereas, each dried chilli samples in solution from had lower intensity than those in a solid form in terms of pungent odour related-attributes, except fresh chilli odour (FO) attribute. This result can be explained by the affinity of the aroma compounds in sample matrix (Kinsella, 1988; Hollowood, 2000). In the present study, the solution samples were prepared by 2% ethanol, which dissolved the pungent odour compound (i.e. 1P3O). Therefore, the compound could be retained in solvent rather than to be released from the sample to the human nose. The aroma compounds perceived by humans must be released from the food matrix for entering airways of the nose and then come into contact with the olfactory receptors (Hollowood, 2000).

In the same form of samples, fresh chilli samples were perceived to be the most FO attribute, while sun dried chilli samples was the least one ($P \leq 0.05$). This could be because of dominant fermented odours in sun dried chilli sample. It is possible that the fermentation was caused by contaminated microorganisms during the exposure to the sun in the open air during the long drying period (Mbugua and Karuri, 1994; Mangaraj *et al.*, 2001). Ground and solution of hot air chilli (HD and SHD) samples presented lower intensity of FO than the ground and solution freeze-dried chilli (FD and SFD) samples do ($P \leq 0.05$). However, the hot air dried chilli samples

had similar intensity of RNO and SPO attributes to freeze dried chilli samples. This is due to the similarity of pungent odour-volatile compounds in the hot air and freeze-dried chilli samples. These compounds are 1-penten-3-one (pungent odour), β-caryophyllene (spicy odour), 2-octanol (spicy odour) and 3-chloro-benzaldehyde (pungent odour) which were identified by GC-MS (the results are also mentioned in Chapter 2).

The hot air dried chilli (HD and SHD) samples had higher intensity of burnt chilli odour (BO) attribute than the freeze dried and sun dried chilli samples, respectively. This could be because of the formation of volatile flavour compounds, namely 2-acetyl furan (licorice odour), furfural (almond odour) and 5-methylfurfural (caramel odour) occurred during hot air drying process (as mentioned in Chapter 2). These compounds are created by the heat temperature of hot air drying, which induces the formation of volatile flavour compounds in a Maillard reaction (Apriyantono and Ames, 1993; Elmore *et al*., 2009). Whereas, the freeze drying is the best drying method for avoiding damage caused by heat, it produces a product with superior physical and chemical qualities of products (Ratti, 2001; Park and Kim, 2007). Therefore, the fresh chilli odour presented in freeze-dried samples with higher intensity than other dried samples. All dried chilli samples contained higher intensity of RNO and SPO than the 0.2 µl/l 1P3O standard sample ($P \leq 0.05$).

In terms of hotness related-attributes, the solution samples presented higher intensity than the ground samples. This may be because that the solution can cover more thoroughly the taste receptors than the solids (Alley and Alley, 1998).

From this study, freeze dried chilli (FD and SFD) sample had the highest score in all hotness related-attributes when compared with other dried chilli samples in the same prepared form, including fresh chilli (F and SF) sample ($P \leq 0.05$). This findings agree with the instrumental results reported in Chapter 2 which were found that the freeze dried chilli sample having the highest capsaicin content (1.29 mg/g; 20,640.54 SHU), then followed by hot air (1.17 mg/g; 18,720.20 SHU), sun dried (0.98 mg/g; 15,680.79 SHU) chilli samples ($P > 0.05$) and fresh chilli (0.58 mg/g; 9,280.84 SHU) ($P \leq 0.05$), respectively. This could be concluded that the perceived hotness in the samples depends on the sample form (solid/solution) and capsaicin content.

3.4.4 Sensory profiling of dried chilli by Principal Component Analysis

Principal Component Analysis (PCA) illustrates an overview of the characteristics of all samples. The PCA illustrations show that three dried chilli samples are dominated with hotness-related attributes. However, the dried samples present less pungent odours than the fresh chilli sample. Burnt chilli odour (BO) attribute mainly is a dominated characteristic of hot air dried chilli. Whereas, dark red colour (DRC) is a unique attribute of sun dried chilli.

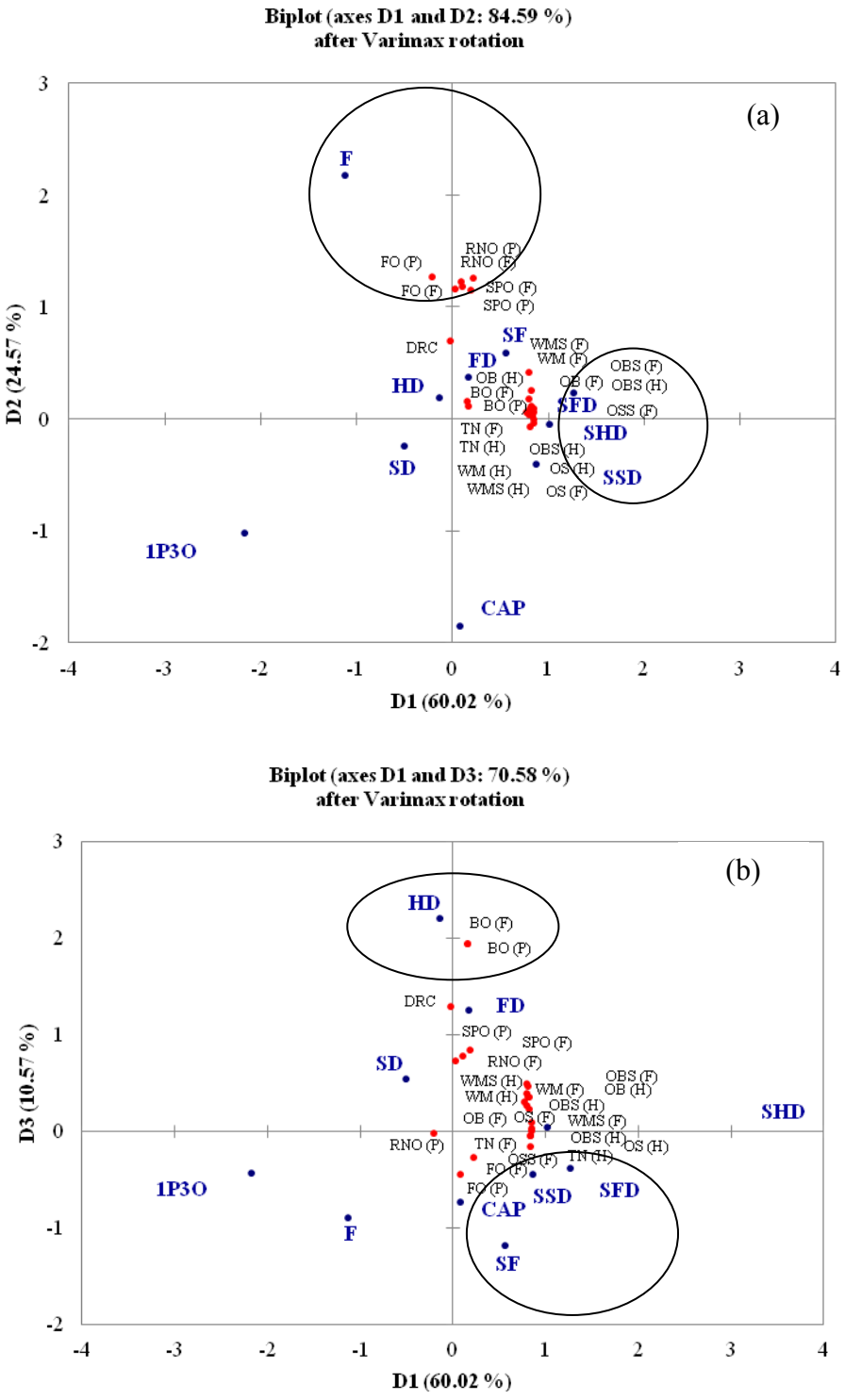
The first three components (PCs1-3) explain 95.15% of the variance in the data set, which means the sensory profiles can be mostly viewed by looking at the plots of PC1-2, PC1-3 and PC2-3. The factor loadings shown in Table 18 and the bi-plot shown in Figure 10 shows four compact groups of samples. The first group situates at positive end of PC1 (dominates by SHD, SFD and SSD), and highly presents OBS, OS, OSS and characteristics in both of hotness and flavour-related intensity assessments. The PC1 explains the hotness related-attribute with accounts for 60.02% of the variation. The second group of samples dominates by F sample in positive side of PC2 and mainly FO, RNO and SPO attributes in both of pungent odour and flavour-related intensity assessments. This component describes the pungent odour related- attributes with accounts for 24.57% of the data variation. The third group locates at positive side of PC3 dominates by HD with the highest values of BO attribute and it accounts for 10.56% of the data variation.

The interpretation of each PC gives an overview of sensory profiles of the experimental samples. The PCA characterises fresh chilli samples with high fresh chilli odour and less hotness related-attributes. Whereas, the hot air and freeze-dried chilli samples are grouped together because of burnt chilli odour, raise-to-nasal pungent odour and sting-pungent odour attributes. Only, the sun dried chilli sample is described as darker red colour and less fresh chilli odour. The three dried chilli samples in solution form are grouped together by hotness-related attributes.

Table 18 Factor loading values of variables on PC1-PC3 of sensory attributes for ground dried chilli, solution dried chilli and standard samples

	PC1	PC2	PC3
Visual			
Dark red colour (DRC)	-0.028	0.525	0.638
Pungent odour intensity (orthonasal perception)			
Burnt chilli odour (BO (P))	0.180	0.115	**0.963***
Fresh chilli odour (FO (P))	0.099	**0.921***	-0.218
Raise-to-nasal pungent odour (RNO (P))	-0.245	**0.952***	-0.006
Sting-pungent odour (SPO (P))	0.220	**0.867***	0.417
Hotness-related intensity (oral perception with nose-clip)			
Warm in mouth (WM (H))	**0.903***	0.044	0.151
Warm in mouth after spitting (WMS (H))	**0.954***	-0.056	0.178
Oral burn (OB (H))	**0.961***	0.083	0.175
Oral burn after spitting (OBS (H))	**0.942***	0.131	0.198
Oral sting (OS (H))	**0.978***	0.050	-0.021
Oral sting after spitting (OSS (H))	**0.987***	0.070	-0.020
Tongue numb (TN (H))	**0.993***	-0.029	0.018
Flavour-related intensity (retronasal perception)			
Burnt chilli odour (BO (F))	0.194	0.083	**0.962***
Fresh chilli odour (FO (F))	0.256	**0.948***	-0.130
Raise- to-nasal pungent odour (RNO (F))	0.028	**0.872***	0.365
Sting-pungent odour (SPO (F))	0.118	**0.889***	0.388
Warm in mouth (WM (F))	**0.933***	0.025	0.246
Warm in mouth after spitting (WMS (F))	**0.932***	0.314	0.135
Oral burn (OB (F))	**0.963***	0.188	0.116
Oral burn after spitting (OBS (F))	**0.949***	0.071	0.231
Oral sting (OS (F))	**0.992***	0.046	0.048
Oral sting after spitting (OSS (F))	**0.982***	0.017	-0.078
Tongue numb (TN (F))	**0.992***	-0.003	0.003
Percentage of total variability	**60.017**	**24.568**	**10.566**
Percentage of accumulated variability	**60.017**	**84.585**	**95.151**

Note: * refers to the positively correlative level of variable on the PC axis.
(P), (H) and (F) refer to pungent odour, hotness and flavour intensity assessment, respectively.



(b)

Figure 10 PCA bi-plot of hotness and pungent odour profile analysed by trained panellists; PC1-PC (a), PC1-PC3 (b)

Note: (P), (H) and (F) refer to pungent odour, hotness and flavour intensity assessment, respectively.

3.4.5 Linkages between sensory descriptive analysis and instrument assessments

Partial Least Squares (PLS) provides the key findings from the instrumental measurements which are linked the sensory attributes perceived by the panellists (Figure 11). The PLS was performed on the 20 volatile compounds derived from the instruments (as reported in Chapter 2) with regard to the intensity of pungent odour and hotness-related attributes, namely fresh chilli odour (FO), burnt chilli odour (BO), raise-to-nasal pungent odour (RNO), sting-pungent odour (SPO), warm in mouth (WM), oral burn (OB), oral sting (OS) and tongue numb (TN). The volatile flavour components derived from SPME method, which can give a good estimation of the aroma profile as well as the perception of the human nose (Brunton *et al*., 2001; Machiels and Istasse, 2003), are regressed with main sensory attributes in order to predict the relationships between the two sets of variables by PLS. In this case, the pungent and hotness-related attributes that contribute to the main pungent and hotness compounds were chosen as Y-variables (dependent variables) and volatile flavour compounds as X-variables (predictors). A bi-plot of the samples and their characteristics was obtained by PLS option in XLSTAT software. The connection of sensory descriptors and volatile compounds in chilli samples testifies the existence of a relationship between variables.

Table 19 Equation of the model from PLS

Equation of the model	Coefficient determination (r^2)
Y_1= 0.285-1.65X_1+0.002X_2-0.009X_3+0.058X_4+0.648X_5+0.765X_6+0.636X7 +0.132X8+1.781X_9+2.375X_{10}+1.018X_{11}+0.383X_{12}+0.170X_{13}-0.449X_{14} -0.035X_{15}+0.046X_{16}+0.020X_{17}+0.007X_{18}+1.531X_{19}-0.020X_{20}	0.993
Y_2 = 0.231+1.499X1+0.001X_2-0.001X_3+0.159X_4+0.159X_5-0.041X_6+0.256X_7 -1.155X_8-0.062X_9-0.110X_{10}-0.660X_{11}+0.207X_{12}-1.540X_{13}+3.332X_{14} +1.033X_{15}+0.125X_{16}+0.016X_{17} +0.032X_{18} -0.420X_{19}+4.137X_{20}	0.655
Y_3 = 0.308-0.839X1+0.001X_2-0.004X_3+0.001X4+0.002X_5+0.841X_6+0.033X_7 +0.622X_8+0.059X_9+0.080X_{10}+0.481X_{11}+0.008X_{12}+1.122X_{13}-0.030X_{14} -0.048X_{15}+0.010X_{16} - 0.001X_{17}- 0.002X18+0.306X_{19} - 0.030X_{20}	0.994
Y_4= 0.506-0.280X_1+1.301X_2-0.003X_3+0.051X_4+0.071X_5+0.012X_6+0.001X_7 +0.388X_8+0.033X_9+0.037X_{10}+0.222X_{11}+0.324X_{12}+0.518 X_{13}+0.473X_{14} +0.036X_{15}+0.039X_{16}+0.004X_{17}+0.008X_{18}+0.141X_{19}+0.023X_{20}	0.996
Y_5= 0.069+3.892X_1+0.001X_2– 0.004X_3+0.150X_4+0.195X_5-0.007X_6+0.530X_7 -0.206X_8+0.003X_9-0.019X_{10} - 0.118X_{11}+0.973X_{12}-0.278X_{13}+2.467X_{14} +0.190X_{15}+0.118X_{16}+0.014X_{17}+0.028X_{18}-0.075X_{19}+0.117X_{20}	0.982
Y_6= 0.295+1.603X_1-0.001X_2+0.001X_3+0.062X_4+0.080X_5-0.025X_6+0.828X_7 -0.694X_8 -0.041X_9 - 0.066X_{10} - 0.397X_{11}+0.401X_{12}-0.925X_{13}+1.480X_{14} +0.114X_{15}+0.049X_{16} +0.007X_{17}+0.013X18-0.252X19+0.070X20	0.861
Y_7= 0.199+1.517X_1+0.001X_2-0.002X_3+0.091X_4+0.118X_5-0.005X_6+0.017X_7 -0.150X_8+0.0003X_9-0.014X_{10}-0.086X_{11}+0.591X_{12}-0.200X_{13}+0.347X_{14} +0.117X_{15}+0.072X_{16}+0.008X_{17}+2.363X_{18}-0.055X_{19}+0.072X_{20}	0.814
Y_8= 0.309+1.096X_1-0.001X_2+0.001X_3+0.045X_4+0.058X_5-0.019X_6+0.009X_7 -0.538X_8-0.032X_9-0.051X_{10}-0.303X_{11}+0.291X_{12}-0.708X_{13}+0.628X_{14} +0.084X_{15}+0.035X_{16}+0.005X_{17}+1.166X_{18}-0.193X_{19}+0.052X_{20}	0.995

Note: Y_1 = Fresh chilli odour, Y_2 = Burnt chilli odour; Y_3 = Raise-to-nasal pungent odour; Y_4 = Sting pungent odour; Y_5 = Warm in mouth; Y_6 = Oral burn; Y_7 = Oral sting; Y_8 = Tongue numb; X_1 = Capsaicin; X_2 = 1P3O; X_3 = Acetic acid; X_4 = 2-Ethyl-1-hexanol; X_5 = 2-Octanol; X_6 = Hexanol; X_7 = 5-Methyl-2-(1-methyl)cyclohexanol; X_8 = 2-Methoxy-phenol; X_9 = Benzenemethanol; X_{10} = 2-Pentyl-furan; X_{11} = N-Hexyl acetate; X_{12} = 2-Furanmethanol-acetate; X_{13} = Hexanal; X_{14} = 5-Methylfurfural; X_{15} = 1,3-Cyclohexadiene-1-carboxaldehyde; X_{16} = 5-Ethyl-undecane; X_{17} = 5-Methyl-undecane; X_{18} = β-Caryophyllene; X_{19} = Trans-anethole; X_{20} = Undecane

Table 19 shows the equations of PLS model for predicting the relationships between the sensory attributes (Y-variables) and volatile compound (X-variable). The PLS results show that the FO and RNO attributes can be predicted by

hexanol, benzenemethanol, 2-pentyl-furan, n-hexylacetate, hexanal and trans-anethole ($r^2 > 0.9$). Acree (2004) and Almonds (2009) stated that hexanol, n-hexylacetate and trans-anethole contribute to perceive a herbal odour, while benzenemethanol contributes to fruity odour. Hexanal is also reported to give a glassy, leafy odour which has been found in fresh chilli (*C. annuum*) (Mazida *et al*., 2005). While 2-pentyl-furan is found to contribute to a green odour in fresh chilli and is significantly decreased after cooking (Srisajjalertwaja *et al*., 2012). The SPO attribute presents highly positive correlation (r^2 =0.996) with 1-penten-3-one which has been described as a pungent odour of fresh chilli (Mazida *et al*., 2005; Azcarate and Barringer 2010; Elmore *et al*., 2009). However, it can be seen from this research that the SPO attribute is negative associated with acetic acid (vinegar-like odour) (r^2 = -0.996). The equation model for explaining the relationship between BO and volatile compounds shows low a goodness of fit statistics (r^2 = 0.655). However, it is worth looking at BO attributes associated by 1,3-cyclohexadiene-1-carboxaldehyde, undecane and 5-methylfurfural which are caramel and herbal odour found in dried chilli derived from hot air drying (as mentioned in Chapter 2). The capsaicin which gives oral hotness sensation (Kobata *et al*., 1998; Bosland, 1996; Walsh and Hoot, 2001) presents a good associated by the OB (r^2 = 0.982) and TN (r^2 = 0.995) attributes. The WM and OS attributes are observed with positive correlation with β-caryophyllene (spicy odour) (r^2 = 0.861 and r^2 = 0.814).

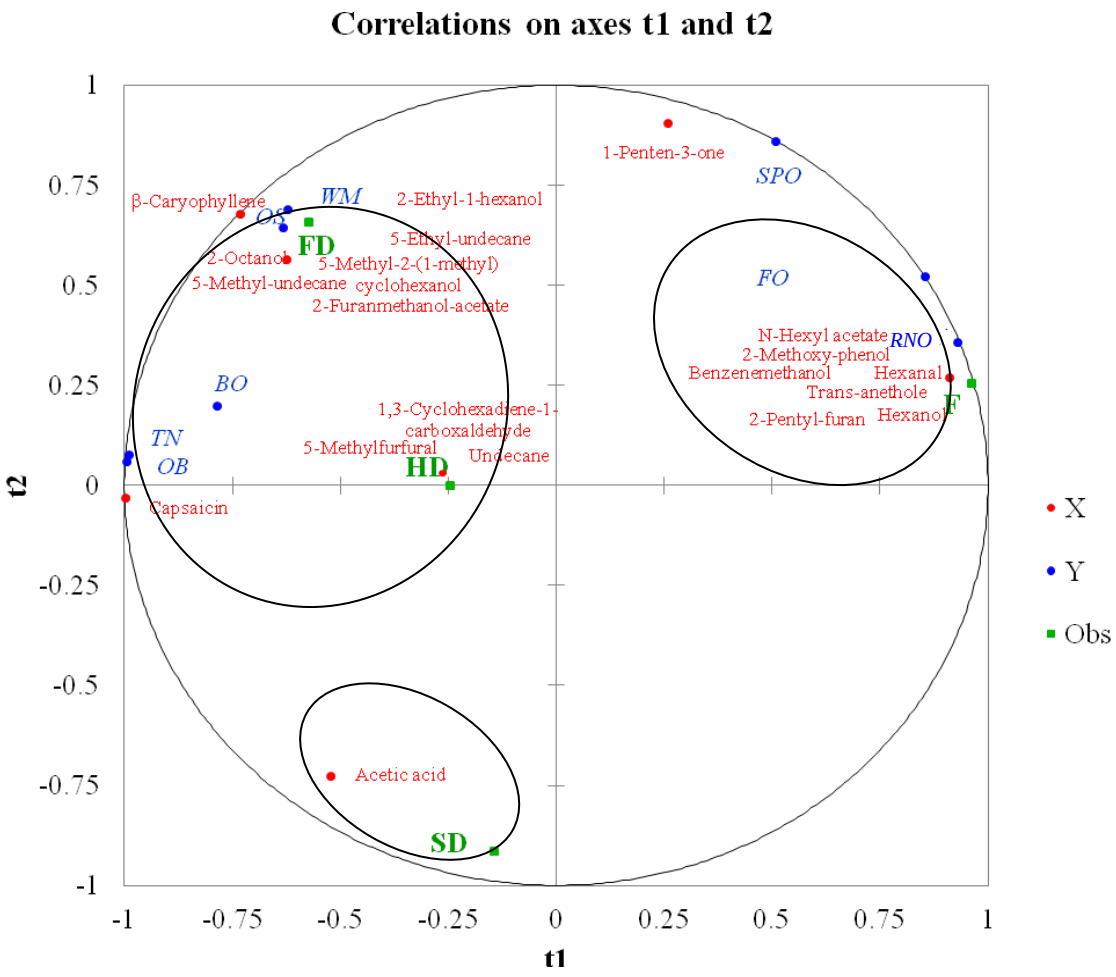


Figure 11 PLS bi-plot of two predicted PCs for sensory attributes (y-variable) and flavour compounds (x-variables) on fresh and dried chilli samples

3.5 Conclusion

Descriptive sensory analysis conducted by the trained panel had delivered sensory profiles of chilli samples. The sensory characteristics of "dark red colour", "burnt chilli odour", "fresh chilli odour", "raise-to-nasal pungent odour", "sting-pungent odour", "warm in mouth", "warm in mouth after spitting", "oral burn, oral", "burn after spitting", "oral sting", "oral sting after spitting" and "tongue numb" were all significant attributes responsible for discrimination among the chilli samples.

Obviously, when the panellists did not wear a nose clip, the retronasal perceptions of hotness-related attributes were definitely stronger or more intense than when they perceived via orthonsal (nose only). All dried chilli samples in solution

form were perceived more intense in pungent odour attributes than those samples presented in a solid form. However, this solid form gave more hotness attributes than the solution form.

PLS technique classified sample characteristics – both sensorial and chemical attributed into three distinctive groups. The first group was of fresh chilli and pungent odour (raise-to-nose) character. The second group was characterised by the drying process such as oral burn, oral sting, warm in mouth, tongue numb and burnt chilli odour which were presented in freeze and hot air dried chilli samples. The last group was a milder characteristic found in sun dried chilli sample such as sting-pungent odour, oral sting and warm in mouth.

Regarding to the sample profiles, the freeze dried chilli contained higher fresh chilli odour and all hotness attributes more than other dried samples. However, freeze and hot air dried samples were not significantly different in terms of raise-to-nasal and sting-pungent odour attributes. Sun dried chilli had intense dark red colour and was perceived the least fresh chilli odour. The research findings derived from sensory descriptive analysis in this chapter are in agreement with the instrumental results reported in Chapter 2 and elsewhere. The 1-penten-3-one and capsaicin are the most dominant compounds to sensorial quality in chilli samples.

CHAPTER 4

INVESTIGATING GROUP THRESHOLDS OF HOTNESS AND PUNGENT ODOUR IN THREE GROUPS OF CHILLI-USERS

4.1 Abstract

Consumer sensitivity to sensorial stimuli can vary substantially based on their food exposures. This chapter aimed to determine recognition thresholds of pungent odour and hotness as well as the principal compounds contributing to pungent odour and hotness of various types of dried chilli. Three different groups of Thai chilli-users were classified, differing by approximate amount of capsaicin regularly consumed as light (<2.19 mg/day), moderate (2.19-4.38 mg/day) and heavy (>4.38 mg/day) users. The major volatile compounds contributing to pungent odour and hotness in chilli were prior identified by Solid Phase Microextraction (SPME) and trained panel. In order to identify recognition thresholds of the two key sensorial attributes by the three groups of chilli-users, an ascending 3-Alternative Forced Choice (3-AFC) (ASTM E697, 2004) was applied. The 3-AFC was conducted with twelve concentrations of sample dilutions in ranges (0.08-16.80 g/l of dried chilli, 0.01-2.04 µl/l of 1-penten-3-one (1P3O) and 0.10-20.16 mg/l of capsaicin), by concentration factor of 1.62. Recognition thresholds were significantly different ($P \leq 0.05$) among the groups on the groups' Best Estimated Threshold (BET). The differences of threshold levels derived from the three groups of chilli-users were supported by a logistic regression approach. Heavy chilli-users presented the highest threshold levels of pungent odours simulated by both dilutions of dried chilli (5.88 g/l) and 1P3O (1.27 µl/l). This group also presented the highest level on hotness thresholds simulated by dilutions of dried chilli (7.19 g/l) and of capsaicin (11.75 mg/l) samples. Whereas the light chilli-users were the most sensitive group and had the lowest thresholds on both attributes. The results demonstrate that exposure to chilli associates with the hotness and pungent threshold levels.

4.2 Introduction

Chilli is widely used as a main spice in various cuisines. Spiciness caused by chilli makes the food more exciting and might furthermore interact with other tastes and odours and enhance overall flavours of food (Prescott *et al*., 1993; Reinbach *et al.,* 2007). Chilli contributes not only to wider range of flavours in foods, but also adds another dimension to meals (Jitbunjerdkul and Kijroongrojana, 2007). The average amount of chilli consumptions per individual person per day were reported to be 2.5 g for an Indian, 5 g for a Thai (Council of Europe, 2001), 7 g for a Korean (Ku and Choi, 1990; Kim *et al*.,2003) and 20 g for a Mexican (Lopez-Carrillo, 1994).

Physiological factors are known to have effects and influenced human sensorial perceptions. Human subjects can individually perceive different intensity level of odours and/or tastes in the same food (Prescott and Stevenson, 1995a; Lawless *et al*., 2000; Reinbach *et al*., 2007). There are reports of decrease in odour and taste perception influenced by age (Cain and Gent, 1991; Schiffman, 1997; Fukunaga *et al*., 2005). Panellists of young age (18-35 years) were reported to have higher sensitivity of four basic tastes (sweet, salty, sour and bitter), odour and a sense of irritation (such as hotness stimulated by capsaicin) than those of older age (36-68 years) (Schiffman and Graham, 2000; Mojet *et al*., 2001; Shusterman *et al*., 2003; Fukunaga *et al*., 2005; Kennedy *et al*., 2010). Thus, young subjects (18-35 years) may be an appropriate choice for panellist recruitment in sensory descriptive and discrimination tests. In the case of gender effects on sensory perception, there was an evidence that females exhibited greater gustatory and olfactory sensitivity than males (Dalton *et al*., 2002). However, gender was not claimed to be the major factor affecting irritants perception ($P>0.05$) (Frot *et al*., 2004; Olofsson and Nordin, 2004). Frequency of chilli consumptions has been reported to have effects on hotness perception (Cowart, 1987; Lawless *et al*., 1985; Lawless *et al*., 2000; Stevenson and Prescott, 1994; Prescott and Stevenson, 1995b; Reinbach *et al*., 2007; Ludy and Mattes, 2012). Non-chilli users (who ate spicy foods < 1 time/month) were reported to perceive burn sensation from a sample of 2.5 to 3 g red chilli in 290 g tomato soup lower than those of chilli-users (who ate spicy foods ≥3 times/week) (Ludy and Mattes, 2012). Eventhough, Lawless *et al*. (2000) reported that there was no

significant difference of sensitivity on hotness level perceived between chilli and non-chilli users, some other researches (Cowart, 1987; Stevenson and Prescott, 1994; Prescott and Stevenson 1995b; Reinbach *et al*., 2007) demonstrated that chilli-users gave weaker oral burn than non-chilli users when the same concentrations of chilli were tested ($P \leq 0.05$) - or in another word, chilli-users are less sensitive to the chilli regarding to the oral burn sensation. Stevenson and Prescott (1994) supported the effects of frequency of chilli consumption on consumers who had repeated exposures to high capsaicin solutions over 2 week-period. The consumers' ratings on hotness intensity then were declined. In addition, individual different preference on spiciness level is proposed to be a possible factor affecting perceived hotness intensity (Prescott and Stevenson, 1995b). Thus, the subjective variables, which are related to the preferred amount and the frequency of chilli consumption, will be included and observed in this study.

In relation to the chemical substances responsible for chilli hotness and pungent odour, capsaicin is a major substance among those found in chilli and causes hotness in mouth (Kobata *et al*., 1998; Bosland, 1996; Walsh and Hoot, 2001). Apart from the capsaicin, 1P3O is found to be a major pungent odour compound in chilli (Luning *et al*., 1995; Van Ruth *et al*., 1995). It produces a strong and sharp pungent odour which is perceived through the nasal cavity (Tainter and Grenis, 2001). Two main chemical compounds were reported to have average detection thresholds, firstly capsaicin is approximately 11.75 ppm in oil and 0.31 ppm in water (Lawless *et al*., 2000) and secondly 1P3O is 0.001 ppm in water (Reineccius and Reineccius, 2002). The smallest concentration level at which an individual subject can perceive and detect as present sensory attribute is a detection threshold whereas recognition threshold is a stimulus makes consciously available and actionable to the consumers (Meilgaard *et al.*, 1999; Lawless and Hayman, 2010).

Measurements of sensory threshold are proposed base on 3-Alternative Forced-Choice (3-AFC) technique by two ASTM International methods (ASTM E679, 2004 and ASTM E1432, 2011). The 3-AFC technique is employed to identify the odd sample in a set of three samples, and can be applied to all three types of thresholds (detection, recognition and different thresholds). For recognition threshold, the detection criteria is the concentration at which the stimulus is correctly identified

(Chambers, 1996). From standard method described in ASTM E679 (2004), a group threshold is calculated from the geometric mean of the panellists' Best Estimated Thresholds (BET) (Cliff *et al*., 2011). The method is recommended to apply with data consisting of 50-100 sets of 3-AFC presentations per individual. The ASTM E679 (2004) method may be considered a shorter method used for estimating sensory thresholds when compares to ASTM E1432 (2011) (Gonzalez- Viñas *et al*., 1998; Eisele and Semon, 2005). ASTM E1432 (2011) suggests the determination of thresholds from the percent of correct response chance of 50% detection level using linear regression. Thus the method of ASTM E1432 (2011) can be applied with data consisting of 20-40 sets of 3-AFC presentations per individual. The ASTM E1432 (2011)'s principle is based on the fact that panellists' answers can be correct through guessing. Nevertheless, there is no published information comparing thresholds of chilli hotness and pungent odour measured by the same set of panellists using the two standard methods.

As hypothesised that consumers can possibly have different perceptual levels in terms of hotness or pungent odour on the same spicy food. Therefore, recognition thresholds of chilli hotness and pungent odour should be tested and estimated in different consumer groups. The estimation of recognition thresholds from various consumer groups can be used as guidelines for food industry, in order to set lowest recognised level of chilli's hotness and pungent odour and also to apply chilli in spicy foods for various target groups of consumers. The standard compounds of capsaicin and 1P3O are applied in this research to account for hotness and pungent odour measurements in chilli. Thresholds are measured based on the two standardised methods (ASTM E679, 2004 and ASTM E1432, 2011) in light, moderate and heavy chilli-users.

4.3 Materials and Methods

4.3.1 Chemicals

Capsaicin (≥95.0%, from *Capsaicum* sp.) and 1P3O (97.0%) were purchased from Sigma-Aldrich Co. LLC. (USA). Ethanol (99%, Food grade) was obtained from LabScan Asia Co., Ltd (Bangkok, Thailand).

4.3.2 Recruitment and classification of consumers

Light (n=40), moderate (n=40) and heavy (n=40) chilli-users whose age were between 18-35 years old, were recruited from 132 candidates. The candidates completed a questionnaire concerning their spicy food consumption (Adapted from Lawless *et al*., 1985) in order to classify them in one of the three user groups. The questionnaire contained 5 consumption-related parts as of;

(i) frequency of consumption of chilli-containing foods,
(ii) amount of chilli contents estimated per dish,
(iii) self-classified hotness level in daily food consumption,
(iv) liking of chilli taste in spicy dish, and
(v) hotness perception tests.

Details of the first 4 parts of the questionnaire are shown in Appendix 4.2. The 5th part was designed to prove the consumer sensitivity in detection of different capsaicin concentrations, and then to classify them into the three groups of chilli users. The hotness perceptual test employed 3-AFC method presenting 12 sets of capsaicin concentrations (0.10, 0.16, 0.26, 0.43, 0.69, 1.12, 1.81, 2.93, 4.74, 7.68, 12.45 and 20.16 mg/l). Each sample set consisted of three samples, including two controls and one target sample. The two controls of the first sample set were 2% ethanol, while the two controls of other sample sets (set 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th, 10th, 11th and 12th) were the next lower concentration of a previous sample set. Details of the 5th part of the questionnaire are shown in Appendix 4.3, Section 4.1.

Consumers were asked to choose only one sample in the set of three that they thought they could notice and detect the hotness differences from the other two samples in the set. Then the results derived from the hotness perceptual test (individual hotness perception), together with the results from questionnaire (frequency and amount of chilli used in consumption, self-classification and liking of chilli) were used to classify the chilli-users into three groups.

4.3.3 Threshold measurement

Ascending 3-Alternative Forced-Choice (3-AFC) method was applied (ASTM E679, 2004) for the threshold measurements. The threshold test on pungent odour was conducted separately from the hotness threshold test. Each set of 1P3O,

capsaicin and ground dried chilli samples was also tested separately on each of three different days. Each set of twelve concentrations in each sample was evaluated starting from low to high concentrations, respectively. Consumers were not told that each sample set were being presented in ascending order of concentrations. All the sample cups were coded with random three-digit numbers. Three samples in a set (two samples of 2% ethanol and one target sample, which was either capsaicin, 1P3O, or dried chilli) were presented at each concentration level, at 25^{o}C. The order of sample presentation was random in each triad of each concentrations presented in every session in order to eliminate positional bias. All the evaluations were in triplicates. Consumers were asked to choose only one sample that they thought they could notice a recognisable taste or odour of the substance among three samples, and give a certainty judgment (guessing or not guessing) on a response sheet (ASTM E679, 2004). Consumers were also asked to differentiate taste or odour of each sample set.

4.3.3.1 Pungent odour threshold test

Samples of 10 ml of 1P3O and dried chilli solutions were presented in covered glass bottles in order to mask the interfering colour and to control any odorant transport. Consumers were instructed to sniff the sample for 5 s, and to rapidly evaluate. Consumers were required to clean their noses between samples by sniffing non scent facial tissue paper before testing the next sample (Reilly and York, 2001). (Appendix 4.3, Section 4.2)

4.3.3.2 Hotness threshold test

Samples of 10 ml of capsaicin and dried chilli solutions were presented in plastic cups. Each sample was presented in red-lighted booth to mask the sample's colour. Consumers were instructed to hold a sample in the mouth for 15 s, expectorate, wait 30 s and then evaluate. After testing the sample, the consumers were also required to rinse their mouths 1 time with sucrose solution (10% sucrose w/w in water) (Nasrawi and Panborn, 1990), 5 times with water and then wait for 5 min between samples (Lawless *et al*., 2000; Allison *et al.*, 1999). (Appendix 4.3, Section 4.1)

4.3.4 Sample screening

The range of sample concentrations for this research chapter derived from preliminary test with a sample of 5 panellists who were chilli-users. This pre-testing was done to ensure that an individual's threshold should fall into neither outside nor near the ends of the range, but well within it. The 1P3O and capsaicin were investigated for pungent odour threshold and hotness testing, respectively. Likewise, solution of dried chilli sample was investigated for both pungent odour and hotness threshold testing. The dried chilli sample which was dried until reach a moisture content of 10-13%, packed in aluminium laminated bags under vacuum condition and then stored at -20^{o}C, as mentioned in Chapter 2 was used in this chapter. The sample was freshly ground just before the use in every session of the entire experiments. Then, the samples were passed to sieve in order to get a typical size of chilli powder (80 meshes), according to Thai Community Product Standard of ground chilli (TCPS 492-2004). The ground sample was mixed with 2% ethanol in order to prepare stock solutions. The mixtures were stirred under room temperature for 10 min, filtered by filter paper No.4, made dilutions and then the solution was subjected to threshold measurements immediately. The concentrations of dried chilli were prepared base on SHU. The hotness levels were achieved by combinations between capsaicin and dihydrocapsaicin contents in the dried chilli and were equal to the hotness level of standard capsaicin. Each sample was served to panellists with an increase concentration level, step by step. Each concentration was tested 5 times by each panellist. The ranges of sample concentrations which were consensually detected and discriminated by the panellists were selected to use in the experiment as shown in Table 20.

Table 20 Concentration of solution for hotness and pungent odour threshold testing

	Preliminary test ranges	**Working test ranges**
Pungent odour of dried chilli (g/l)	0.01-25.00	0.08-16.80
Pungent odour of 1P3O (µl/l)	0.002-5.00	0.01-2.04
Hotness of dried chilli (g/l)	0.01-25.00	0.08-16.80
Hotness of capsaicin (mg/l)	0.03-25.00	0.10-20.16

4.3.5 Sample preparation

A series of dilution was prepared by increasing the concentration of each sample (1P3O, capsaicin and ground dried chilli samples) at a concentration

factor of 1.62 (ASTM E679, 2004) as shown in Figure 12. All serial dilutions were chosen based upon preliminary work that provided a reasonable bracketing of threshold and approximately equal ranges of perceived hotness and pungent odour.

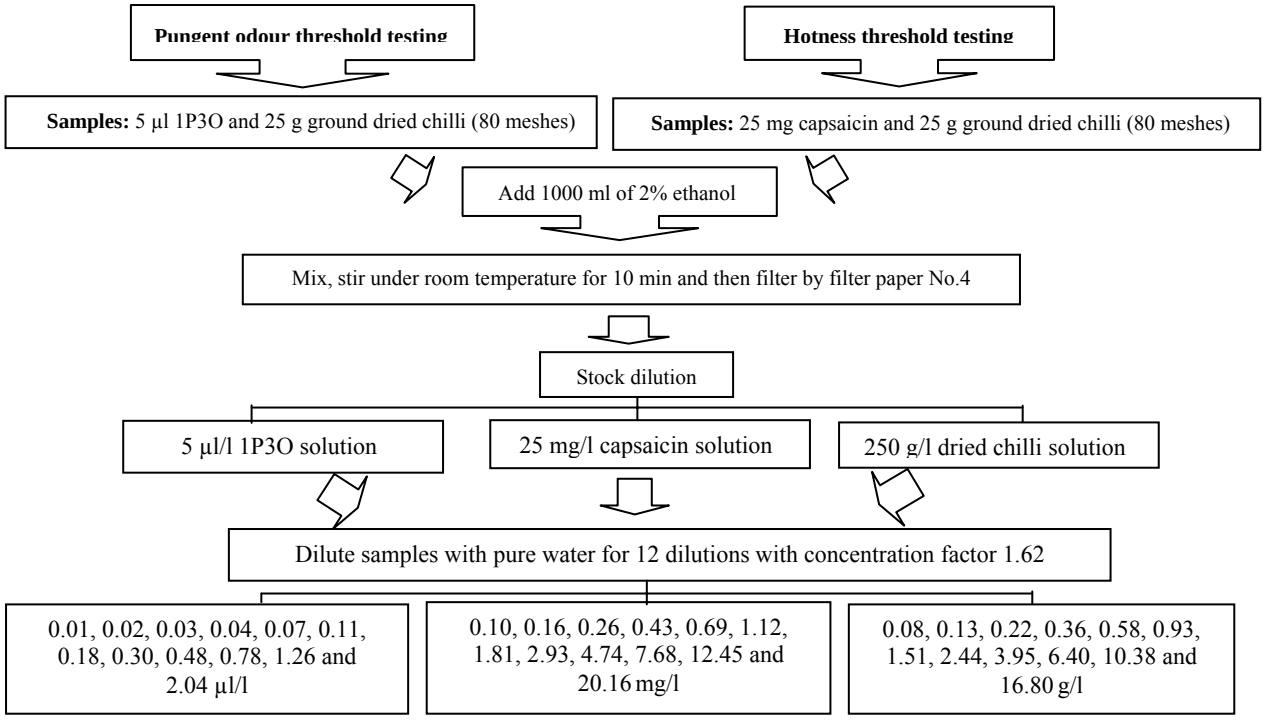


Figure 12 Flow diagram of sample preparation for threshold tests

4.3.6 Calculations of group threshold levels

The recognition thresholds of pungent odour and hotness derived from three consumer groups were calculated by two approaches; Geometric means (ASTM E679, 2004) and Logistic regression (ASTM E1432, 2011).

4.3.6.1 Geometric means

The geometric mean is a measure of central tendency calculated by multiplying a series of numbers and taking the n^{th} root of the product, where 'n' is the number of items in the series (Markowitz, 2012), as following Eq. 1.

$$\text{Geometric mean} = \sqrt[n]{A_1 \cdot A_2 \cdot \ldots A_n}. \qquad \text{(Eq. 1)}$$

Where A_1, A_2 and etc. represent the individual data points, and n^{th} is the total number of data points used in the calculation.

The American Society for Testing and Materials (ASTM E679, 2004) recommends using geometric means for calculating the individual Best Estimated Threshold (BET) and group thresholds (ASTM E679, 2004). In this research, the BET was taken as the geometric mean of the highest concentration missed (or incorrect)

and the next higher concentration. The threshold of each consumer group was the geometric means of the BET of all consumers in each group. The individual consumer group's variation was reported by the standard deviation $\log_{10}$ value (ASTM E679, 2004).

4.3.6.2 Logistic regression

Logistic regression is employed to predict the probability of correct choices by 3-AFC method (ASTM E1432, 2011). Logistic regression is applied to measure relationships between a categorical dependent variable and predictor variables by converting the dependent variable to probability scores (Agresti, 2002). In this study, the sample concentrations of hotness and pungent odour of dried chilli, hotness of capsaicin and pungent odour of 1P3O were predictor variables. A correct identification of an odd sample was the predicted outcome (the dependent variable). The threshold levels for logistic regression method were determined by converting the percentage correct (% correct) for each concentration of each sample to the percentage correct response chance (% correct response chance) using Abbott's formula (Eq. 2) (Aardt *et al*., 2001; Lawless and Heymann, 2010) and by plotting the percentage correct response chance against concentration.

$$\%\ \text{correct above chance} = 100 \times \frac{(\%\ \text{correct} - \%\ \text{correct by chance})}{(100 - \%\ \text{correct by chance})} \qquad \text{(Eq. 2)}$$

Logarithmic trend lines were fitted with data using MS Excel. This result was an equation in the form $Y = m \ln (X) + c$ for determining thresholds. Theoretically, the thresholds are usually determined at the probability of a 50% detection level. For 3-AFC test with a probability of 33.3% correct by chance, 66.7% of identifications is thus required to answer correctly to obtain a true proportion detecting of 50% (Lawless and Heymann 2010; Cliff *et al*., 2011). The correct identification is calculated as shown in Eq. 3.

$$50 = 100 \times (Y - 33.3)/(100 - 33.3) \qquad \text{(Eq. 3)}$$
$$Y = 66.7$$

4.3.7 Statistical analysis

The characteristics of consumer groups were presented with analysis of variance (ANOVA) for quantitative data. The original thresholds obtained by ASTM E679 (2004) procedure (BET values) were not normally distributed, thus the BET values were log-transformed prior to performing ANOVA (Cliff *et al*., 2011). The thresholds obtained by ASTM E1432 (2011) procedure were taken as arithmetic means of the threshold results in triplicates for each consumer group. The thresholds from both methods were presented for the three consumer groups across samples. The experimental designs were a Randomized Complete Block Design (RCBD). Analysis of variance (ANOVA) was also employed to evaluate the effects on individual consumer groups. Significant differences between means were estimated by Duncan's new multiple range test (DMRT), with a level of significance of 0.05. Statistical analyses were performed by using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA, 2002).

4.4 Results and Discussion

4.4.1 Consumer classification

4.4.1.1 Characteristics of consumer groups

Based on the results from the questionnaire (Appendix 4.2 and Appendix 4.3), three different groups of chilli-users had the characteristics as shown in Table 21. Heavy chilli-users (n=40) were a group of consumers who consumed chilli-containing foods at least everyday (mode = 6), liked chilli taste quite a lot (score 7.45/9) and used chilli content at a range of 4.25- 9.71 g/dish. However, heavy chilli-users had the least discriminating ability of hotness to the capsaicin stimuli. This result was agreed with Lawless *et al*. (2000), who found that chilli users rated high scores of chilli questionnaire on chilli liking and frequency of eating spicy foods, but these were contrary with rated threshold score. They indicated that higher chilli-users were associated with lower intensity perception. It was also noted that people who consumed hot and spicy foods on a regular basis were partially desensitized to the sensory effects of oral capsaicin. Hence, these people have much less responsive above the threshold (Prescott and Stevenson, 1995b). Prescott (1999) suggested that chronic desensitisation by capsaicin might produce chronic decrements in taste or

flavour intensity, which could explain why more eaters of chilli had poor sense in hotness discrimination. Furthermore, the moderated and heavy chilli-users were defined by chilli consumption at least 3-4 times a week, which perhaps mean that the sufficient capsaicin exposure in their diet induced the desensitisation (Reinbach *et al*., 2007).

Table 21 Characteristics of chilli-user groups

	Light chilli-users (n=40)		**Moderate chilli-users (n=40)**		**Heavy chilli-users (n=40)**	
	Mode	**Frequency (%)**	**Mode**	**Frequency (%)**	**Mode**	**Frequency (%)**
Frequency of consumption of chilli-containing foods (score 1-7)	2	88.57	4	40.00	6	82.86
Self-classified hotness level in daily spicy food intake (score 1-3)	1	68.57	2	82.86	3	57.14
Capsaicin hotness perception testing (3-AFC) (score 0-12, reported as mode of correct response (%))	85.71	75.00	58.33	80.65	41.63	80.00
	Mean score		**Mean score**		**Mean score**	
Amount of chilli content estimation per meal (capsaicin content (mg/g))	0.49±0.34[c]		1.03±0.58[b]		1.71±0.42[a]	
Liking of chilli flavour in some spicy dish (score 1-9)	3.80±0.91[c]		6.53±0.75[b]		7.45±0.94[a]	

Note: Different superscripts within a row show significant difference (P≤0.05).

4.4.2 Recognition threshold values

The recognition thresholds of each consumer who received 12 sets of 3-AFC presentation were estimated. An example of the estimation capsaicin recognition threshold of heavy chilli-users is shown in Table 22. In the rightmost column in the Table 22, the individual BET was tabulated according to ASTM E679 (ASTM E679, 2004), as the geometric mean of the last missed concentration and the next (adjacent) higher concentration. The geometric mean was determined by taking the arithmetic mean of the BET values and then taking the antilog of that mean (Lawless, 2013). The bottom row shows the tabulation of proportions correct obtained at each level which was in ascending order from the least to the highest capsaicin concentrations. The proportions of correct response were used to predict a recognition

threshold concentration by applying logistic regressions according to ASTM E1432 (2011). The proportions of correct response were then plotted and fitted to a logistic regression equation for predicting the individual recognition threshold as displayed in Figure 13. The cut-off probability of detection of the stimulus (threshold) was determined at 66.7% of correct identification. The average in each group threshold was calculated from the individual threshold inside each group of chilli-users (ASTM E1432, 2011).

Table 22 Capsaicin hotness threshold perceived by heavy chilli-users

Chilli-users	Concentration (mg/l)												BET	Log (BET)
	0.10	**0.16**	**0.26**	**0.43**	**0.69**	**1.12**	**1.81**	**2.93**	**4.74**	**7.68**	**12.45**	**20.16**		
1	0	0	+	0	0	+	0	+	+	+	+	**0**	25.66	1.41
2	0	+	0	0	+	0	0	0	**0**	**+**	+	+	6.03	0.78
3	0	+	+	0	0	+	0	+	**0**	**+**	+	+	6.03	0.78
4	0	0	0	+	+	0	0	0	+	+	+	**0**	25.66	1.41
5	0	+	0	+	0	+	0	+	0	0	0	**0**	25.66	1.41
6	0	0	0	0	+	0	0	0	+	0	+	**0**	25.66	1.41
7	0	+	0	0	0	0	0	+	+	0	**0**	**+**	15.84	1.20
8	+	0	0	0	+	+	+	0	**0**	**+**	+	+	6.03	0.78
9	0	+	0	0	+	+	0	+	+	+	**0**	**+**	15.84	1.20
10	0	0	+	0	+	0	0	0	0	0	**0**	**+**	15.84	1.20
11	+	+	0	+	0	+	0	+	+	+	**0**	**+**	15.84	1.20
12	0	0	0	+	0	+	+	+	+	0	**0**	**+**	15.84	1.20
13	0	+	+	0	0	+	0	0	+	+	0	**0**	25.66	1.41
14	0	+	0	0	+	+	+	+	+	+	+	**0**	25.66	1.41
15	0	0	+	0	0	+	0	0	+	+	**0**	**+**	15.84	1.20
16	0	0	0	+	0	0	+	+	+	+	+	**0**	25.66	1.41
17	0	0	0	+	0	+	+	+	+	0	**0**	**+**	15.84	1.20
18	0	0	0	0	+	0	+	0	+	0	+	**0**	25.66	1.41
19	0	+	0	0	0	0	0	0	+	0	**0**	**+**	15.84	1.20
20	0	0	+	0	+	+	+	+	**0**	**+**	+	+	6.03	0.78
21	+	0	0	0	+	0	+	0	**0**	**+**	+	+	6.03	0.78
22	0	0	+	0	+	+	+	+	+	+	**0**	**+**	15.84	1.20
23	0	+	0	0	+	0	0	+	+	0	**0**	**+**	15.84	1.20
24	+	0	0	+	0	0	+	+	0	+	+	**0**	25.66	1.41
25	0	0	+	+	0	+	+	0	**0**	**+**	+	+	6.03	0.78
26	0	0	+	0	+	0	+	+	**0**	**+**	+	+	6.03	0.78
27	0	0	0	+	0	+	0	+	+	0	0	**0**	25.66	1.41
28	0	+	+	0	0	0	0	+	+	0	0	**0**	25.66	1.41
29	0	+	0	+	0	+	+	0	**0**	**+**	+	+	6.03	0.78
30	0	0	0	0	+	+	+	+	**0**	**+**	+	+	6.03	0.78
31	0	0	0	+	+	+	0	+	+	**0**	**+**	+	9.78	0.99
32	0	0	+	0	0	+	+	+	+	**0**	**+**	+	9.78	0.99
33	0	0	0	+	0	0	0	0	+	**0**	**+**	+	9.78	0.99
34	+	0	+	0	+	+	+	+	**0**	**+**	+	+	6.03	0.78
35	0	0	0	+	0	0	+	0	**0**	**+**	+	+	6.03	1.41
36	+	0	+	0	0	0	+	+	**0**	**+**	+	+	6.03	0.78
37	+	0	+	+	0	+	+	0	**0**	**+**	+	+	6.03	0.78
38	0	0	0	0	+	0	+	0	+	0	+	**0**	25.66	1.41
39	0	0	0	+	0	0	+	0	**0**	**+**	+	+	6.03	0.78
40	0	0	0	0	+	0	+	0	**0**	**+**	+	+	6.03	0.78
													$\Sigma\log_{10}$	45.78
						Group BET, geometric mean							12.30	1.09
						Log_{10} standard deviation								0.26
Proportions correct	19.05	28.57	33.33	38.10	45.24	52.38	54.76	57.14	57.14	61.90	61.90	71.43		

Note: "0" indicates the chilli-user selected any one wrong sample of triad. "+" indicates that the chilli-user selected any one correct sample.

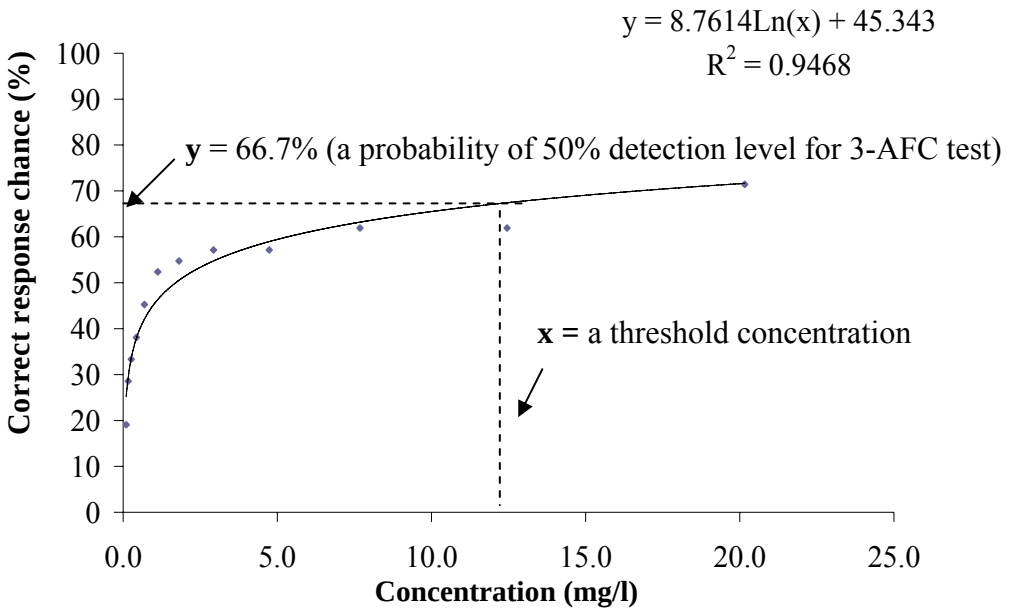


Figure 13 Recognition threshold prediction of hotness in capsaicin samples by heavy chilli-users using logistic regression

4.4.2.1 Geometric mean thresholds

The geometric mean thresholds for all samples of light, moderate and heavy chilli-users were calculated from individual BETs of each group as shown in Table 23. The geometric mean thresholds show significant variations among the different consumer groups ($P \leq 0.05$). The ANOVA and Duncan's multiple-range test of the geometric mean threshold reveals that the recognition thresholds on dried chilli pungent odour, 1P3O pungent odour, dried chilli hotness and capsaicin hotness of the heavy chilli-users were higher than that of other groups ($P \leq 0.05$).

Table 23 Geometric means of hotness and pungent odour threshold among three groups of chilli-users

	Geometric mean of group threshold*		
	Light chilli users (n=40)	**Moderate chilli-users (n=40)**	**Heavy chilli users (n=40)**
Pungent odour of dried chilli (g/l)	0.61 ± 0.04^{c}	1.68 ± 0.04^{b}	5.76 ± 0.40^{a}
Pungent odour of 1P3O (µl/l)	0.04 ± 0.01^{c}	0.20 ± 0.05^{b}	1.27 ± 0.30^{a}
Hotness of dried chilli (g/l)	0.58 ± 0.06^{c}	2.16 ± 0.04^{b}	7.07 ± 0.33^{a}
Hotness of capsaicin (mg/l)	0.87 ± 0.53^{c}	2.09 ± 0.43^{b}	11.75 ± 0.28^{a}

Note: Different superscripts within a row show significant difference ($P \leq 0.05$).

* Geometric mean ± Log_{10} standard deviation.

4.4.2.2 Logistic regression thresholds

Figure 15 shows the chance (probability) of receiving correct response on pungent odour and hotness thresholds of 1P3O, capsaicin and dried chilli. The percentage of correct response increased with increasing concentration. The regression model was fitted on probability of correct response to obtain an equation for the calculation of group recognition thresholds (Tables 24). ASTM E1432 (2011) recommended a probability of 66.7% correct identification in 3-AFC for threshold specification when logistic regression is used. Therefore, the concentration specified at the detection level was reported as a recognition threshold.

The heavy chilli-users detected low sensitivity of pungent odour in dried chilli. The logistic regression predicts that recognition threshold of pungent odour of dried chilli is at a concentration of 5.88 g/l (Figure 14a). However, the logistic regression predicts that recognition threshold of pungent odour of 1P3O samples is at a concentration of 1.34 µl/l (Figure 14b). Moderate and light chilli-users correctly identified the pungent odour of 1P3O samples at a concentration of 0.23 µl/l and 0.06 µl/l, respectively as shown in Figure 3b. In addition, logistic regression predicts that the recognition thresholds of hotness (in dried chilli samples) derived from heavy chilli-users (7.19 g/l) are higher than the moderate (2.23 g/l) and light (0.58 g/l) chilli-users, respectively (Figure 14c). Furthermore, the hotness threshold of heavy chilli-users on capsaicin samples are reported at the highest concentration (12.79 mg/l) and are followed by moderate (2.36 mg/l) and light (0.96 mg/l) chilli-users, respectively (Figure 14d).

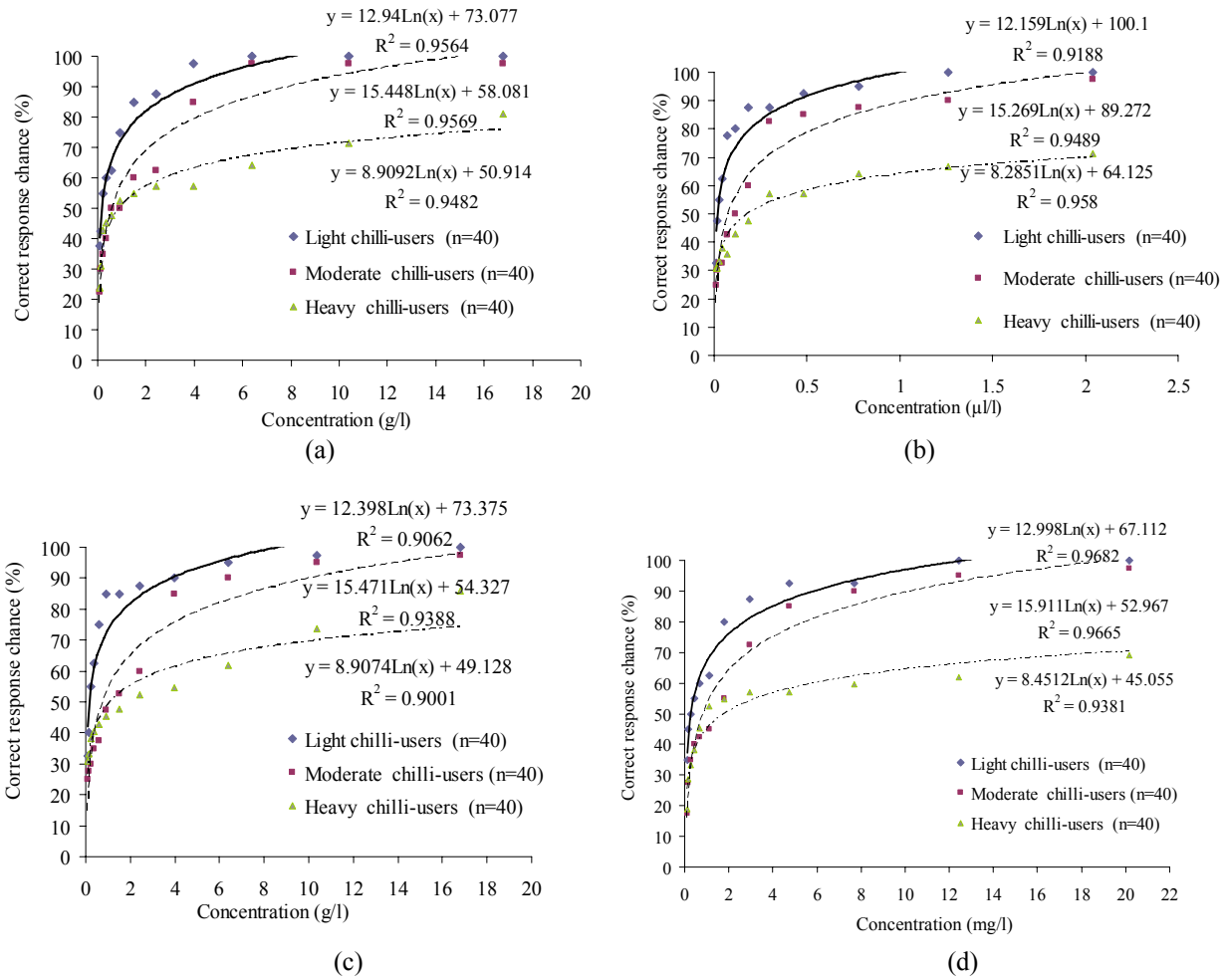


Figure 14 Comparison of correct response chance on dried chilli pungent odour (a) 1P3O odour (b), dried chilli hotness threshold (c) and capsaicin hotness (d) among three groups of chilli-users

Table 24 Predicted concentration using logistic regression of hotness and pungent odour threshold among three groups of chilli-users

	Group threshold * at *p(x)* = 0.667		
	Light chilli-users (n=40)	**Moderate chilli-users (n=40)**	**Heavy chilli-users (n=40)**
Pungent odour of dried chilli (g/l)	0.61 ± 0.08^{c}	1.75 ± 0.18^{b}	5.88 ± 0.17^{a}
Pungent odour of 1P3O (µl/l)	0.06 ± 0.02^{c}	0.23 ± 0.09^{b}	1.34 ± 0.92^{a}
Hotness of dried chilli (g/l)	0.58 ± 0.09^{c}	2.23 ± 0.14^{b}	7.19 ± 0.20^{a}
Hotness of capsaicin (mg/l)	0.96 ± 0.38^{c}	2.36 ± 1.17^{b}	12.79 ± 1.78^{a}

Note: Different superscripts within a row show significant difference ($P\leq0.05$).

*Calculated using $p(x) = a\ ln(x)+b$ from logistic regression when $p(x) = 0.667$.

In summary, results from both methods showed that heavy chilli-users had the highest threshold levels of pungent odours simulated by dried chilli at concentration levels of 5.76 g/l from geometric mean and of 5.88 g/l from logistic regression method. Both methods also delivered the highest threshold levels of pungent odour stimulated by 1P3O dilutions (1.27 µl/l from geometric mean and 1.34 µl/l from logistic regression methods) derived from the heavy chilli-users. Regarding to the hotness perception, the heavy chilli-user group had the highest threshold levels of hotness simulated by dried chilli with concentrations of 7.07 g/l (by geometric mean) and 7.19 g/l (by logistic regression), as well as stimulated by capsaicin dilutions of 11.75 mg/l (by geometric mean) and 12.79 mg/l (by logistic regression). This pronounces that the level of thresholds in hotness and pungent odour are to be considered in relation to the amount and frequency of chilli consumption. Similar to this finding are well corresponded to the heavy chilli-users who accustomed to consume high frequency and amount of chilli were not sensitive in detecting hotness and pungent odour. For example, whilst Thai light chilli-users who held the lowest group thresholds, were sensitive to the stimuli representing hotness at 0.96 mg/l capsaicin level, and to pungent odour at 0.06 µl/l 1P3O level, the stimuli concentrations of Thai lowest threshold levels are yet higher than hotness thresholds reported in European (0.08 mg/l capsaicin) (Schneider *et al*., 2014) and Japanese consumers (0.70 mg/l capsaicin) (Fukunaga *et al*., 2005). In addition, Thai moderate chilli-users shared similar range of capsaicin threshold level with Turkish consumers (1.53 mg/l capsaicin) (Mavi *et al*., 2000) (Table 25). The consumers who consume chilli more frequently are likely to be induced to develop sense adaptation more than the consumers who consume it less frequently (Helson, 1964; Stevenson and Prescott, 1994). Therefore, they are not very sensitive to the stimuli. It is also possible that in some cases, the repeated exposure by regular consumption of high amount of chilli may cause damage to the unmyelinated nerve receptors and fibres, and lead to chronic desensitisation (Duner-Engstrom *et al*., 1986; Stevenson and Prescott, 1994; Prescott and Stevenson, 1995b; Prescott, 1999), hence results in poor ability of judgement in hotness and pungent odour perceptions.

Table 25 Literatures of threshold measurements

Study design	Group threshold	Reference
Study: hotness recognition threshold **Subjects:** 10 American panellists **Treatments:** serial dilutions of 0.06, 0.09, 0.12, 0.18, 0.3, 0.50 and 0.70 mg/l capsaicin **Methods:** 2-AFC (% correct response), paired sample of one target and one water sample **Test condition:** 10 ml samples, swirled in mouth for 10s, expectorated, evaluated, rested for 5 min, rinsed mouth using water	**Recognition threshold:** 0.18 mg/l capsaicin	Sizer and Harris, 1985
Study: hotness detection threshold **Subjects:** American panellists, 11 non chilli-users and 20 chilli-users (18-35 years old) **Treatments:** serial dilutions of 0.031, 0.063, 0.125, 0.250 and 0.500 mg/l capsaicin in water base **Methods:** 3-AFC (BETs), triad sample of one target and two water samples **Test condition:** 15 ml samples, swirled in mouth for 5s, expectorated, evaluated, rested for 5 min, rinsed mouth using water	**Detection threshold:** 0.30 mg/l capsaicin for non-chilli users and 0.34 mg/l capsaicin for chilli-users (P>0.05)	Lawless *et al*., 2000
Study: hotness detection threshold **Subjects:** Turkish panellists, 14 females (19-23 years old and 24 males (18-25 years old) **Treatments:** range from 0.24 to 15.44 mg/l capsaicin **Methods:** (3-AFC) (BETs), triad sample of one target and two water samples **Test condition:** 1 ml samples, swirled in mouth for 5s, expectorated, evaluated, rested for 30 s, rinsed mouth using water	**Detection threshold:** 1.53 mg/l capsaicin	Mavi *et al*., 2000
Study: hotness recognition threshold **Subjects** Japanese panellists, 30 young (18-29 years old) **Treatments:** serial dilutions of 0.03, 0.09, 0.15, 0.31, 0.91, 1.53 and 3.05 mg/l capsaicin **Methods:** (3-AFC) (BETs), triad sample of one target and two water sample **Test condition:** held a paper-disk soaked in capsaicin solution on the tip of the tongue for 10 s, removed, evaluated, rinsed mouth using water	**Recognition threshold:** 0.70 mg/l capsaicin	Fukunaga *et al*., 2005
Study: hotness detection threshold in different bases of oil and water **Subjects:** 21 European panellists (students) **Treatments:** Serial dilutions of 0.0223, 0.045, 0.090, 0.180 and 0.360 mg/l capsaicin in water base, and serial dilutions of 0.150, 0.450, 1.350 and 4.050 mg/l capsaicin in oil base **Methods:** 3-AFC (BETs), triad sample of one target and two control samples (water or sunflower oil) **Test condition:** 5 ml samples, swallowed, evaluated, rinsed mouth using water	**Detection threshold:** 0.080 mg/l capsaicin in water base and 0.826 mg/l capsaicin in oil base	Schneider *et al*., 2014

4.4.3 Comparisons of thresholds from Geometric means and Logistic regression

The geometric mean calculation gave slightly lower level of thresholds when compared with the thresholds predicted from logistic regression, for all samples across all chilli-user groups (Tables 26-28). In light chilli-users, the geometric mean method gave 0-33.30 % lower in threshold concentrations than that of logistic regression method. Whereas, the range of 3.13-15.25% was the different yield between recognition threshold levels derived from the geometric mean and logistic regression methods, measured by moderated chilli-users. The geometric mean method gave 1.00-8.10% lower threshold than another method. This result was in line with the work from Senthil and Bhat (2011) and Cliff *et al*. (2011). They compared geometric mean and logistic regression methods for testing cardamom aroma in different media (Senthil and Bhat, 2011), and testing sulphur compounds in different base wines (Cliff *et al*., 2011). Both reports concluded that the geometric mean method yielded lower thresholds than the logistic regression method. However, in order to appropriately compare the results from both methods, it was suggested to determine and consider the probabilities of correct responses as well. The probability of correctly responses by geometric mean method can be obtained by replacement of x value in a logistic regression equation by the geometric mean thresholds. If the obtained percentage of geometric mean method is very near 66.7% correct response, it means that the results of the both methods are very similar. According to the determination, the results of this research clearly show probabilities of all recognition threshold levels are well closed to 66.7% correct response. Therefore, this indicates that the threshold levels determined by logistic regression are quite similar to the thresholds levels determined by geometric mean calculation.

Table 26 Comparison between geometric mean and logistic regression of hotness and pungent odour threshold in light chilli-users

	Group threshold [a] at *p(x)* = 0.667	**Geometric mean of group threshold [b]**	**Probability of group threshold (%) [c]**
Pungent odour of dried chilli (g/l)	0.61	0.61	66.68
Pungent odour of 1P3O (µl/l)	0.06	0.04	65.89
Hotness of dried chilli (g/l)	0.58	0.58	66.53
Hotness of capsaicin (mg/l)	0.96	0.87	65.30

Note: [a] Calculated using $p(x) = a\ ln(x)+b$ from logistic regression, when $p(x) = 0.667$. [b] Calculated using geometric mean. [c] Calculated using $p(x) = a\ ln(x)+b$ when using x = group thresholds obtained from geometric mean approach.

Table 27 Comparison between geometric mean and logistic regression of hotness and pungent odour threshold in moderate chilli-users

	Group threshold [a] at *p(x)* = 0.667	**Geometric mean of group threshold [b]**	**Probability of group threshold (%) [c]**
Pungent odour of dried chilli (g/l)	1.75	1.68	66.09
Pungent odour of 1P3O (µl/l)	0.23	0.20	64.70
Hotness of dried chilli (g/l)	2.23	2.16	66.24
Hotness of capsaicin (mg/l)	2.36	2.09	64.69

Note: [a] Calculated using $p(x) = a\ ln(x)+b$ from logistic regression, when $p(x) = 0.667$. [b] Calculated using geometric mean. [c] Calculated using $p(x) = a\ ln(x)+b$ when using x = group thresholds obtained from geometric mean approach.

Table 28 Comparison between geometric mean and logistic regression of hotness and pungent odour threshold in heavy chilli-users

	Group threshold [a] at *p(x)* = 0.667	**Geometric mean of group threshold [b]**	**Probability of group threshold (%) [c]**
Pungent odour of dried chilli (g/l)	5.88	5.76	66.51
Pungent odour of 1P3O (µl/l)	1.34	1.27	66.11
Hotness of dried chilli (g/l)	7.19	7.07	66.55
Hotness of capsaicin (mg/l)	12.79	11.75	65.86

Note: [a] Calculated using $p(x) = a\ ln(x)+b$ from logistic regression, when $p(x) = 0.667$. [b] Calculated using geometric mean. [c] Calculated using $p(x) = a\ ln(x)+b$ when using x = group thresholds obtained from geometric mean approach.

The correlations between the geometric mean and logistic regression methods for the group thresholds of light, moderate and heavy chilli-users were shown highly significant correlations (r = 0.999 P≤0.01) (Figure 15). This notion was in agreement with the work of Cliff *et al*. (2011). The report presented strong correlation between the geometric mean and logistic regression methods (r=0.949 at P≤0.001). The highly significant correlation (P≤0.01) of thresholds between the two methods found in this research indicates that both methods are validated and can be used to calculate the group thresholds for 1P3O pungent odour, dried chilli pungent odour and capsaicin hotness and dried chilli hotness.

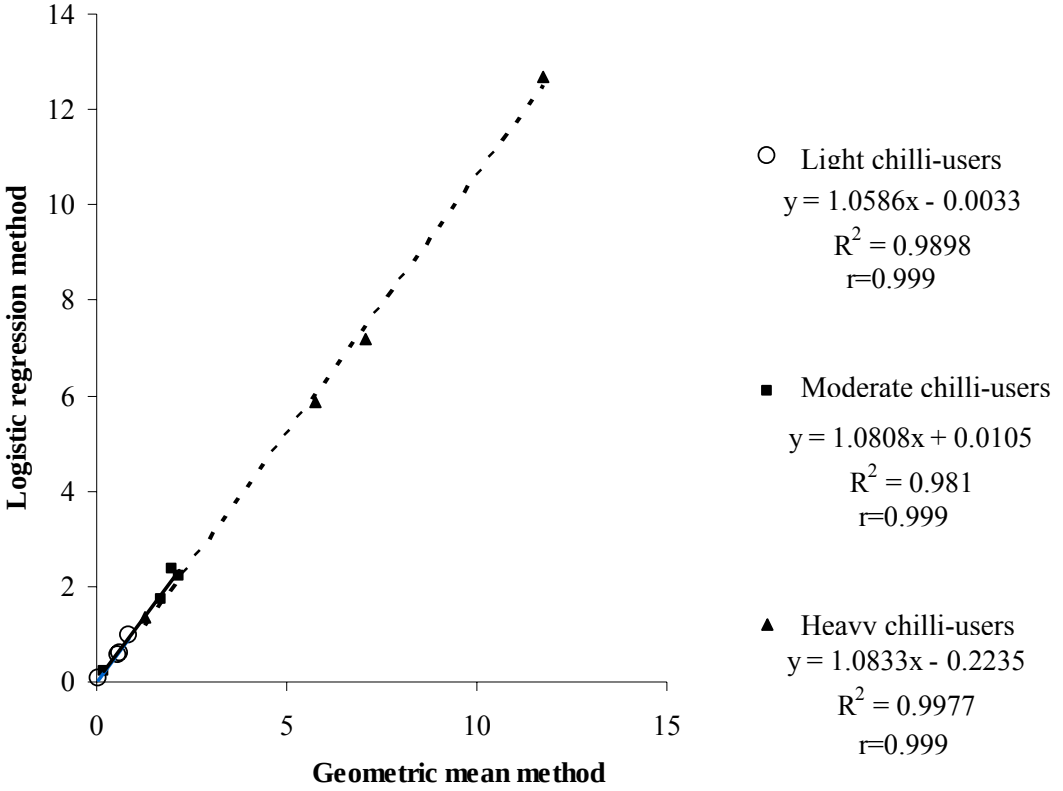


Figure 15 Correlation between geometric mean and logistic regression methods among three groups of chilli-users

However, there was a precaution when the geometric mean method was applied. As threshold levels derived from the method can be easily influenced by incorrect responses by consumers as a result of fatigue or sensory adaptation. Because there is a finite probability that a correct answer will occur by chance alone. It is important that a consumer continues to take the test until there is no doubt by that

person of the correctness of the choice (Lawless, 2010). Therefore, logistic regression might be a preferable method in measuring consumer's threshold because it does not solely rely on individual responses (Senthil and Bhat, 2011).

4. 5 Conclusion

Three groups of Thai chilli-users were grouped by approximate amount of capsaicin regularly consumed in their diet, into light (<2.19 mg/day), moderate (2.19-4.38 mg/day), and heavy (>4.38 mg/day) users. The hotness and pungent odour recognition thresholds of chilli consumer groups were present in great ranges. Heavy chilli-users had the highest group threshold of the hotness and pungent odour. Their threshold levels of hotness and pungent odour simulated by dried chilli were approximately 9-13 times higher than the group thresholds of light chilli-users, and about 3-4 times that of moderate chilli-users. In terms of simulations by the standard solutions i.e. capsaicin hotness and 1P3O pungent odour, the heavy chilli-users showed their group thresholds approximately 13-32 times greater than the light users and about 5-6 times of the moderate group thresholds. When compared the Thai group thresholds found in this research with the thresholds reported elsewhere, the lowest recognition threshold levels of Thais were higher than that of European (detection threshold) and Japanese (recognition threshold) consumers. The capsaicin hotness threshold level of Thai moderate users was similar to the detection threshold of Turkish consumers. It evidently points out that intense and repeated exposure plays major role in human sense perception and sensitivity. Regarding the threshold measurement methods, the group thresholds derived from ASTM E679 (2004) using BET calculation were well correlated and in line with the ones derived from ASTM E1432 (2011) by logistic regression.

CHAPTER 5

ASSESSING HEDONISM OF HOTNESS AND PUNGENT ODOUR INTENSITIES IN PROCESSED DRY CHILLI SAMPLES

5.1 Abstract

Light (n=40), moderate (n=40) and heavy chilli-users (n=40) were recruited based on criteria of capsaicin intakes in their spicy food diets and age group in order to measure their liking on three types of dry chilli samples. Hedonism on pungent odour, hotness and flavour of hot air (HD), freeze (FD) and sun (SD) dried chilli samples were assessed by the three user groups using 9 point-hedonic category scale. HD sample was most liked across three groups of chilli-users ($P \leq 0.05$). The hotness liking scores were measured in HD samples and in standard compounds such as capsaicin and 1-penten-3-one (1P3O). Each group of chilli-users was presented with the samples contained capsaicin concentrations near the group's thresholds. This chapter concludes that individual group of users liked the hotness and pungent odour at a middle level of the group threshold bracket. The concentrations of hotness and pungent odour of dried chilli which were most liked, are 0.58 and 0.61 g/l in light chilli-users, 2.23 and 1.75 g/l in moderate chilli-users, and 7.19 and 5.88 g/l in heavy chilli-users.

5.2 Introduction

Chilli (*Capsicum* spp.) is appreciated for its hotness, pungent odour, taste and aroma. It is applied in foods for food additive, pigment and physiological and pharmaceutical uses (Cisneros-Pineda *et al.*, 2007). It is a common spice in Thai cuisine and it is widely consumed as a food component throughout the world, particularly in South East Asia and Latin-American countries (Laohavechvanich *et al.*, 2006). There is an evidence of an increasing interest in dried chilli for both the local market and foreign market (Hossain and Bala, 2007). Chilli is one of economic plants and involves with Thai society, especially with its daily cooking. Generally, it is mostly consumed in the form of dried powder or fine flake as a condiment (Turhan

et al., 1997). Fresh chilli is normally preserved by drying immediately after harvest to obtain the dried chilli, to decrease overflow of fresh chilli in the market and also to control the market price (Charmongkolpradit *et al*., 2010). The most conventional drying method applied to chilli is sun drying. The duration of sun drying which is required to reduce moisture content in fresh chilli, depends on the quality of sunlight, temperature and air humidity. Hence the sun dried chilli present various moisture content levels between and within processing batches. In addition, they might be contaminated with dust, dirt, rainfall, animals, birds, rodents, insects and microorganisms (Mangaraj *et al*., 2001). Alternatively, thermal drying method such as hot air drying has been popularly applied due to its short drying time, uniform heating and more hygienic characteristics (Chung *et al*., 1992). The other option in producing dried chilli is using freeze drying. It is claimed to be the best process for retention dried chilli quality (Park and Kim, 2007), however it is also the most expensive process for drying (Ratii, 2001).

Certainly, not only colour of the dried chilli product is a concern issue, but hotness and volatile compounds (i.e. perceived pungent odour) of dry chilli are also important attributes when applied dry chilli in food products (Lease and Lease, 1962; Pordestimo *et al*., 2004; Luning *et al*., 1995; Govindarajan, 1986; Venskutonis, 1997; Lin and Durance, 1998; Szumny *et al*., 2010). The hotness compounds such as capsaicin has been found to be exposed to greater thermal and oxidative degradation. Thus the drying temperature also affects on the levels of capsaicin available in chilli (Pordestimo *et al*., 2004). Likewise, the other major volatile compounds such as pungent odour (i.e. 1P3O) can be decomposed during drying process (Luning *et al*., 1995). As far as dried chilli quality concerns, the differences in hotness and pungent odour compounds in dried chilli can alter consumer choice. Decompositions of the compounds influence on different sensorial property perceived by consumers and may not meet the requirement of the consumers in terms of flavour attributes. Therefore, an appropriate drying method of chilli will be specified based on consumer liking scores on both of the hotness and pungent odour attributes.

Hotness is a sensation but it is not classified as a taste in the technical sense, because the sensation does not arise from taste buds and a different set of nerve fibres carries its signal to the brain. The hotness perception pathway starts from the

stimulation of somatosensory fibres on the tongue. It can be defined as the trigeminal sensations perceived during tasting (Green and Hayes, 2004; Delwiche, 2004). Pungent odours such as an irritant odour elicit activities in both olfactory and trigeminal chemoreceptors and create sensations of stinging (Delwiche, 2004). The stinging sensations involve the course of volatiles through nasal passages located in the nose, when a person inhales them (Meilgaard *et al*., 1999).

There are several factors reported to influence consumer liking on hotness and pungent odour. Byrnes and Hayes (2013) mentioned a number of factors such as social influences, repeated exposure to capsaicin, physiological differences in chemosensation and personality, were likely to have an effect on consumer liking of capsaicin-containing foods. However, a strong relationship was found between consumer liking of spicy foods and frequency of chilli consumption (Byrnes and Hayes, 2013; Ludy and Mattes, 2012). In the case of novel product, it is also well established that repeated experience with unfamiliar foods increases liking (Pliner, 1982; Birch, 1999). This is supported by Stevenson and Yeomans (1995) who found that there was a linear increase in rated liking for the hotness between the first and the fifth exposure to a ratatouille contained either 2.5 or 5.0 mg/l capsaicin.

Rosin (1990) suggested that the preference for chilli develops as a benign form of sensation or thrill seeking. Other evidence suggests that the repeated exposure to capsaicin reduces overall sensitivity (Green and Rentmeister-Bryant, 1998), perhaps promoting its liking and long-term use. This shift in sensitivity could partially explain why habitual regular chilli consumers rate the hotness of capsaicin less intense than do non-chilli consumers (Lawless *et al*., 1985). It is also possible that individuals vary in their liking of spicy foods (Tepper, 2006). Bear in mind, however, the perception and hedonism of hotness or spiciness in humans are subjected to the amount of the effective compounds that stimulate hotness. Gradual introduction of increasing spiciness in foods has demonstrated to reverse the initial dislike and induce strong preferences for the burn, flavour, and aroma of spicy foods (Logue and Smith, 1986; Rozin and Schiller, 1980). However, it has also been proposed that frequent exposure to capsaicin and chilli can result in chronic desensitization (Cowart, 1987; Karrer and Bartoshuk, 1991; Lawless *et al*., 1985; Stevenson and Prescott, 1994). The effect is partially responsible for the variation in reported sensitivity and liking of the

hotness of capsaicin. In summary, chilli liking is not merely a case of increased tolerance with repeated exposure, but rather that there is an affective shift towards a preference for hotness that is not found in chilli dislikers (Rozin and Schiller, 1980; Stevenson and Yeomans, 1993).

There have been some examinations of hedonic responses to hotness between consumers who were relatively naïve to chilli with those who frequently used and liked it, and the results are reported that regular chilli-users (who ate spicy foods ≥3 times/week) have higher liking than non-chilli users (who ate spicy foods < 1 time/month) (Rozin and Schiller, 1980; Rozin, 1990; Byrnes and Hayes, 2013; Ludy and Mattes, 2012). The pungent odour, however, has not been investigated in this regard. This study has taken the gap and opportunity to research more into the effects of familiarity and amount of capsaicin and 1P3O on consumer liking. It is anticipated that the results may help product developers to set attractive levels of hotness and pungent odour in mild, moderate and very spicy food products, corresponding to the preferences of different consumer groups.

The aim of this present study is to determine consumer liking on hotness and pungent odour attributes. The research was conducted to measure liking scores of the three consumer groups on two sets of samples (three dried chilli products -SD, HD and FD; and ascending concentration series of dried chilli, capsaicin and 1P3O.

5.3 Materials and Methods

5.3.1 Chemicals

Capsaicin (≥95.0%, from *Capsaicum* sp.) and 1P3O (97.0%) were purchased from Sigma-Aldrich Co. LLC. (USA). Ethanol (99%, Food grade) was obtained from LabScan Asia Co., Ltd (Bangkok, Thailand).

5.3.2 Recruitment of consumers

People have different preferences on spiciness or hotness levels of their food. Therefore, food producers usually consider formulating their spicy products (e.g. chilli sauce, chilli paste and savoury snacks) in various hotness levels like mild, moderate and very spicy in order to cover the range of consumer acceptance. The different preferences of people may be depended on personal ability**,** and preferred

amount and frequency of chilli consumption (Bartoshuk *et al*., 2004; Reckitt Benckiser plc, 2013). Bartoshuk *et al*. (2004) reported that there were three groups of tasters: supertasters who endured the most intense taste sensations, medium tasters who perceived intermediate taste intensities and non-tasters who perceived the weakest taste intensities. In addition, Reckitt Benckiser plc (2013) divided consumers into the light, moderate and heavy users according to their frequency of consumption spicy food. With this reason, this study classified consumers into three groups of chilli user, namely light, moderate and heavy chilli users. One hundred and thirty two chilli consumers were pre-recruited using public advertisements. The screened and recruited subjects were: 1) in age group between 18-35 years old, 2) non-smokers, 3) willing to taste spicy samples, 4) not allergic to the test samples and compounds, and 5) in good health. After screening, 120 participants completed a questionnaire of spicy food consumption (Appendix 4.2 and Appendix 4.3, Section 4.1), concerning their spicy food perception and experience (According to the recruitment and classification of consumers in Chapter 4). The three groups of chilli consumers were divided as light (n=40), moderate (n=40) and heavy (n=40) chilli-users based on their frequency of consumption on chilli-containing foods and their preference in hotness.

The heavy chilli-user group consisted of 25 females and 15 males whose age ranged from 20 to 34 years. They were persons who ate chilli- containing foods every day, liked chilli very much (score 7.45/9) and used chilli in a range of 4.25-9.71 g/dish. Moderate chilli-user group consisted of 28 females and 12 males whose age ranged from 19 to 35 years. They were persons who ate chilli more than once a week. Their average chilli liking score was ‘liked moderately’ (score 6.59/9). They used chilli in a range of 2.12-4.25 g/dish. Light chilli-user group consisted of 29 females and 11 males whose age ranged from 20 to 35 years. They were persons who ate spicy food less than once a month. Their average chilli liking score was rather dislike (score 3.80/9). The average chilli content used in diet was less than 2.12 g/dish.

5.3.3 Measurement of overall consumer liking in pungent odour, hotness and flavour of dried chilli samples

This experiment aimed to study liking of consumer in typical features attributed from dried chilli. Dried chilli was chosen as it is typically sold and consumed in both Thai and foreign markets. Hot air (HD), freeze dried (FD) and sun dried (SD) chilli samples which were dried until reach a moisture content of 10-13%, packed in aluminium laminated bags under vacuum condition and then stored at -

20^{o}C, as mentioned in Chapter 2 were used in this chapter. Samples were freshly ground just before use in every session of the entire experiments. Then, the samples were passed to sieve in order to get a typical size of chilli powder (80 meshes), according to Thai Community Product Standard of ground chilli (TCPS 492-2004). Two and a half grams of the three samples, i.e. FD, HD and SD were presented in clear plastic containers to all consumers (light, moderate and heavy chilli-users). Each sample was separately served monadically by random order. The pungent odour, hotness and flavour liking measurements were conducted using procedures mentioned in the assessments of consumer liking. (Appendix 4.6, Section 4.6.1)

Dried chilli sample which obtained the highest liking score was selected to be tested for further the experiment in the Topic 5.3.4.

5.3.4 Measurement of overall consumer liking in relation to specific threshold intensities of pungent odour and hotness

5.3.4.1 Preparation of experimental samples

The samples were consisted of standard 1P3O, capsaicin and dried chilli solutions. The dried chilli solutions were prepared at the concentrations based on hotness levels - SHU of capsaicin standard, combining concentrations of capsaicin and dihydrocapsaicin contents in the dried chilli, as mentioned in the results of Chapter 2. All samples were mixed with 2% ethanol in order to prepare stock solutions. The mixtures were stirred by clean stirring rod for 10 min, filtered by filter paper No.4, diluted with pure water and then submitted to the liking test immediately.

The liking scores of both pungent odour and hotness were observed in dried chilli solutions. Liking scores of pungent odour in standard 1P3O and hotness in standard capsaicin solutions were also separately measured. In this experiment, the panellists rated their liking on hotness attributes via mouth without nose-clips. (Appendix 4.6, Section 4.6.2)

The samples used in this experiment were divided into 2 sample sets, as following:

5.3.4.1.1 Dried chilli samples, 1P3O and capsaicin were prepared in 5 concentrations (the 1st sample set) which covered the ranges of hotness and pungent odour threshold levels of each consumer group (as mentioned in Chapter 4). The three

different sets of 5 samples would be served to each of the three groups. All sample concentrations are shown in Table 29.

5.3.4.1.2 Five concentration levels of dried chilli samples (the 2nd sample set), 1P3O and capsaicin which covered the ranges of hotness and pungent odour threshold levels of the three consumer groups were prepared (the threshold levels were prior identified in Chapter 4). Hence, the same set of 5 samples were be presented to all consumer groups. All sample concentrations are shown in Table 30.

Table 29 Sample concentrations for consumer liking measurement in relation to threshold intensities of pungent odour and hotness in the 1st set

Consumer groups	**Samples**			
	Pungent odour of dried chilli (g/l)	**Hotness of dried chilli (g/l)**	**Pungent odour of 1P3O (µl/l)**	**Hotness of capsaicin (mg/l)**
Light chilli-users	0.23	0.22	0.02	0.37
	0.38	0.36	0.04	0.59
	0.61	0.58	0.06	0.96
	0.99	0.94	0.10	1.56
	1.60	1.52	0.21	2.52
Moderate chilli-users	0.67	0.85	0.09	0.90
	1.08	1.38	0.14	1.46
	1.75	2.23	0.23	2.36
	2.84	3.61	0.37	3.82
	4.59	5.85	0.60	6.19
Heavy chilli-users	2.24	2.74	0.51	4.87
	3.63	4.44	0.83	7.90
	5.88	7.19	1.34	12.79
	9.53	11.65	2.17	20.72
	15.43	18.87	3.52	33.57

Table 30 Sample concentrations for consumer liking measurement in relation to threshold intensities of pungent odour and hotness in the 2nd set

Samples			
Pungent odour of dried chilli (g/l)	**Hotness of dried chilli (g/l)**	**Pungent odour of 1P3O (µl/l)**	**Hotness of capsaicin (mg/l)**
0.38	0.36	0.04	0.59
0.61	0.58	0.06	0.96
1.75	2.23	0.23	2.36
5.88	7.19	1.34	12.79
9.53	11.65	2.17	20.72

The 5 sample solutions, 10 ml each sample, were individually presented to, light, moderate and heavy chilli-users. The 1P3O and dried chilli samples were prepared and presented in covered glass bottles for hedonic measurement of pungent odour and in a plastic cup for hotness liking.

5.3.5 Assessment of consumer liking

All consumers were presented with random 3-digit coded samples. Each sample was separately served with balance first-order and carry-over-effect design (MacFie *et al*., 1989) (Figure 16). The samples were presented and assessed in red masking light in a sensory booth to reduce colour interference effects. Consumers were asked to evaluate their liking on pungent odour, hotness and flavour attributes of samples, respectively.

In the evaluation of pungent odour, each sample was presented in a covered opaque glass bottle for masking any interference of colour and appearance, and for controlling the transfer of any odourants. The consumers were required to clear their nasal cavities with soft tissue papers between samples (Adapted from Cometto-Muñiz *et al.,* 2000). An interstimulus interval of 5 min was allowed to permit the pungency to subside. For determining hotness and flavour liking, the samples were presented in plastic cups. The consumers were required to rinse their mouths once with sucrose solution (10% sucrose w/w in water) (Nasrawi and Panborn, 1990), 5 times with water and then wait for 5 min between samples (Lawless *et al*., 2000; Allison *et al.*, 1999). An interstimulus interval of 5 min was allowed to permit any residual to subside. The likings of all sensory attributes were scored on 9-point hedonic category scale. The methods of assessment for each sensory attributes are shown in Table 31.

(a) (b)

Figure 16 Sample presentation for consumer liking test; hotness and flavour liking (a), pungent odour liking (b)

Table 31 Method of assessment pungent odour, hotness and flavour liking

Tested sensory attributes	Method of assessment
Pungent odour	Assess liking on sharp sensation in nose, sniff and hold the breath 3-5 s
Hotness	Assess liking on hotness sensation in mouth when wear nose-clips, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s
Flavour	Assess liking on hotness sensation in mouth when not wearing nose-clips, hold sample in mouth 15 s with minimize movement, expectorate and wait 30 s

5.3.6 Statistical analysis

The experimental designs of experiments 5.3.2 and 5.3.3.1.2 were 3x5 Factorial designs (3 group consumers x 5 sample concentrations). The experimental design of experiment 5.3.3.1.1 was a Randomized Complete Block Design (RCBD). Data were subjected to analysis of variance (ANOVA). Duncan's new multiple range test (DMRT), with a level of significance of 0.05. Statistical analyses were performed by using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA, 2002). Principal Component Analysis (PCA) was applied to observe relationships among consumer liking scores in both hotness and pungent odour attributes by XLSTAT software (XLSTAT Pro 2008).

5.4 Results and Discussion

5.4.1 Measurement of overall consumer liking on pungent odour, hotness and flavour on dried chilli samples

The mean scores and ANOVA results of the liking on pungent odour, hotness and flavour in the three dried chilli samples were reported in Table 32. A fixed model of two-way ANOVA (samples and chilli consumer groups) with interaction was applied to analyse the liking scores obtained from the 3 attributes. Significant differences of liking between samples were detected in all attributes ($P \leq 0.05$). There was no effect of chilli consumer groups and no interaction effect between samples x chilli consumer groups in all cases ($P > 0.05$). There was no significant difference in liking scores derived from three consumer groups on an individual sample on all attributes ($P > 0.05$).

In general, SD was the least liked sample on all attributes across 3 groups of consumers. HD received the highest liking scores on all attributes, but is not significantly different from FD ($P > 0.05$). There was no significant difference in hotness liking of the three samples with similar range of hotness levels (SD = 0.98 mg/g, 15,680.79 SHU; HD = 1.17 mg/g, 18,720.20 SHU; FD = 1.29 mg/g, 20,640.54 SHU) among the consumer groups.

Light chilli-users (n=40) gave the highest liking scores of pungent odour and flavour attributes on HD with a range of 5.57-5.65. Moderate and heavy chilli-users (n=40) also liked pungent odour of HD most. The liking scores of hotness and flavour of HD and FD samples were not significantly different by all three consumer groups.

It can be concluded that there was an agreement of liking among all consumer groups (n=120). They disliked pungent odour, hotness and flavour attributes of SD. This may be caused by the least fresh chilli odour remained in SD, as mentioned in Chapter 3. However, the consumers showed a tendency of liking in all attributes of HD. This may be due to familiarity of similar type of HD samples commonly produced, used and sold in Thailand. Most of dried chilli products available in Thai retailers are produced by conventional hot air drying. Heath *et al.* (2011) stated that the familiarity with taste, odour and flavour of food played role in consumers' liking. This is supported by Pliner (1982) who used unfamiliar tropical

fruit juices (e.g. guava and mango juices) and presented them to participants 0, 5, 10, or 20 times. This research was reported that an increased familiarity led to increased liking. In addition, Pliner (1982) also noted that the familiarity led to increases in liking after a week delay. Likewise, Birch and Marlin (1982) invested the familiarity effect on food liking for two-year-old children. As their parents would predict, children preferred sweeter foods and foods which they were familiar with. This may be a well-known psychological effect that people express liking for things merely because they are familiar with them (Bornstein and Crave-Lemley, 2004). It may not only be familiarity, but also a positive experience with a product lead to increased favourability. It is intuitive findings that peoples consider there are things that they used to like or things that they have come to like over time (Hoeffler *et al*., 2013).

Table 32 Mean liking scores and ANOVA of pungent odour, hotness and flavour in the dried chilli samples

Attributes	Samples	Liking scores			P-value		
		Light chilli-users (n=40)	Moderate chilli-users (n=40)	Heavy chilli-users (n=40)	Samples	Chilli consumer groups	Chilli consumer groups x Samples
Pungent odour	SD	$4.20\pm1.60^{b,A}$	$4.65\pm1.65^{b,A}$	$4.10\pm1.68^{b,A}$	0.000	0.062	0.917
	FD	$5.38\pm1.75^{a,A}$	$6.00\pm1.69^{a,A}$	$5.58\pm1.89^{a,A}$			
	HD	$5.65\pm1.73^{a,A}$	$6.08\pm1.52^{a,A}$	$5.95\pm1.71^{a,A}$			
Hotness	SD	$4.95\pm1.72^{b,A}$	$4.65\pm1.60^{b,A}$	$5.35\pm1.89^{b,A}$	0.000	0.219	0.191
	FD	$5.35\pm1.90^{a,A}$	$6.10\pm1.33^{a,A}$	$5.93\pm2.00^{a,A}$			
	HD	$5.57\pm1.63^{a,A}$	$6.10\pm1.50^{a,A}$	$5.68\pm2.04^{a,A}$			
Flavour	SD	$4.15\pm1.83^{b,A}$	$3.93\pm1.54^{b,A}$	$4.57\pm2.05^{b,A}$	0.000	0.648	0.317
	FD	$5.50\pm1.88^{a,A}$	$6.13\pm1.32^{a,A}$	$5.88\pm2.28^{a,A}$			
	HD	$5.75\pm1.61^{a,A}$	$5.90\pm1.90^{a,A}$	$5.55\pm1.99^{a,A}$			

Note: Different small superscripts within dried chilli samples of each attribute refer to the significant difference ($P\leq0.05$)

Different capital superscripts among consumer groups in each attribute refer to the significant difference ($P\leq0.05$).

5.4.2 Measurement of consumer liking in relation to threshold intensities of pungent odour and hotness

In this part, the HD was selected as a representative of dried chilli samples because it received the highest liking scores of all attributes. The three groups of consumers rated their liking of the research samples (solutions of chilli and standards) by pungent odour, hotness and flavour attributes.

5.4.2.1 Measurement of overall consumer liking by the 1st sample set

The individual consumer group evaluated their liking in pungent odour and hotness of the assigned samples. The concentrations of capsaicin in the samples were calculated and selected to fit within their threshold bracket. The average liking scores of hotness, pungent odour and hotness attributes received from each consumer group were shown in Table 33. The results showed that the intensity affected significantly on liking score of all attributes ($P \leq 0.05$), in all chilli-user groups. The middle dried chilli, capsaicin and 1P3O concentrations of each sample set received the highest liking scores from the individual consumer groups with a range of 6.08-6.58 (moderately liking level). Interestingly, the intensities of capsaicin/1P3O in most liked samples were also matched with the group thresholds of the individual groups.

Pungent odour of dried chilli, at middle concentrations of each intensity range (0.61 g/l for light, 1.75 g/l for moderate, and 5.88 g/l for heavy chilli-users) - were most liked by all consumer groups. Approximately equal quantities of 1P3O in these dried chilli concentrations were 0.0012, 0.0037 and 0.012 µl/l, respectively (Based on Chapter 2 results). Pungent odour of 1P3O solutions were also most liked at middle concentration of each groups (0.06 µl/l for light, 0.23 µl/l for moderate, and 1.34 µl/l for heavy chilli-users). Although, the most liked dried chilli has lower contents of 1P3O compound than concentrations of most liked 1P3O sample, their liking pattern were alike. The difference in liked concentrations may be influenced by other minor pungent odour compounds such as 3-Chloro-benzaldehyde. They naturally exist in dried chilli and may intensify the perceived pungent odour in dried chilli samples. Hotness was most liked at middle concentrations of dried chilli and capsaicin solutions in all consumer groups. The most liked capsaicin concentrations from the two sample types are comparable similar.

Table 33 Liking mean scores of varied sample intensities from the three consumer groups (the 1st sample set)

Attributes	Light chilli-users (n=40)		Moderate chilli-users(n=40)		Heavy chilli-users (n=40)	
	Intensities	Liking scores	Intensities	Liking scores	Intensities	Liking scores
Pungent odour of dried chilli (g/l)	0.23	5.68±1.27^{bc}	0.67	5.28±1.74^{b}	2.24	5.58±1.52^{b}
	0.38	6.03±1.42^{abc}	1.08	5.40±1.81^{b}	3.63	5.70±1.59^{b}
	0.61	6.50±1.22^{a}	1.75	6.33±1.61^{a}	5.88	6.58±1.03^{a}
	0.99	6.12±1.38^{ab}	2.84	5.95±1.74^{ab}	9.53	5.95±1.57^{b}
	1.60	5.48±1.74^{c}	4.59	5.50±1.47^{b}	15.43	5.60±1.66^{b}
Pungent odour of 1P3O (µl/l)	0.02	4.92±1.44^{b}	0.09	5.58±1.63^{b}	0.51	5.27±1.57^{bc}
	0.04	5.10±1.15^{b}	0.14	5.48±1.30^{b}	0.83	5.43±1.63^{bc}
	0.06	6.43±1.12^{a}	0.23	6.08±1.12^{a}	1.34	6.25±1.37^{a}
	0.10	5.25±1.30^{b}	0.37	5.75±1.55^{b}	2.17	5.60±1.26^{b}
	0.16	4.78±1.56^{b}	0.60	4.90±1.60^{c}	3.52	4.90±1.61^{c}
Hotness of dried chilli (g/l)	0.22	5.18±1.65^{c}	0.85	4.75±1.97^{c}	2.74	5.15±1.79^{d}
	0.36	5.10±1.79^{c}	1.38	5.60±1.68^{b}	4.44	5.67±1.56^{c}
	0.58	6.40±1.34^{a}	2.23	6.10±1.66^{a}	7.19	6.08±1.44^{a}
	0.94	5.85±1.59^{b}	3.61	5.90±1.57^{a}	11.65	5.82±1.62^{b}
	1.52	4.75±1.88^{d}	5.85	5.35±1.58^{bc}	18.87	4.85±1.96^{e}
Hotness of capsaicin (mg/l)	0.37	5.20±1.20^{cd}	0.90	5.15±1.72^{c}	4.87	5.08±1.73^{b}
	0.59	5.85±1.37^{b}	1.46	5.65±1.55^{b}	7.90	5.10±1.71^{b}
	0.96	6.38±1.13^{a}	2.36	6.20±1.24^{a}	12.79	6.25±1.10^{a}
	1.56	5.65±1.49^{bc}	3.82	5.88±1.16^{a}	20.72	5.58±1.50^{b}
	2.52	4.88±1.49^{d}	6.19	5.15±1.09^{c}	33.57	4.98±1.64^{b}

Note: Different superscripts within each attribute in a column refer to the significant difference among different intensities ($P \leq 0.05$).

5.4.2.2 Measurement of overall consumer liking by using the 2nd sample set

To ascertain that light, moderate and heavy chilli-users liked different intensities of hotness and pungent odour, a set of similar sample intensities was evaluated by all three groups of chilli consumers. The results are shown in Tables 34-35.

Table 34 ANOVA of liking of hotness and pungent odour in a similar sample set (the 2nd sample set)

Source of variance	P-value			
	Pungent odour of 1P3O	**Hotness of capsaicin**	**Pungent odour of dried chilli**	**Hotness of dried chilli**
Intercept	0.000	0.000	0.000	0.000
Chilli consumer groups	0.054	0.440	0.767	0.634
Sample intensities	0.000	0.000	0.005	0.003
Chilli consumer groups x Sample intensities	0.000	0.000	0.004	0.000

The ANOVA results showed significant difference of liking scores between sample intensities ($P \leq 0.05$). The interaction effect between sample and consumer groups was found to be significant in all attributes ($P \leq 0.05$) (Table 34). In other words, the heavy users liked higher levels of hotness (e.g. capsaicin 12.79 mg/l; 204,640 SHU) and of pungent odours (e.g. IP3O 1.34 µl/l) than the moderate (e.g. capsaicin 2.36 g/l; 37,760 SHU, 1P3O 2.23 µl/l) and light chilli-users (e.g. capsaicin 0.96 g/l; 15,360 SHU, 1P3O 0.06 µl/l) (Table 35).

To illustrate the liking patterns of all samples by individual chilli consumer groups, the data were analyzed by PCA. The PCA bi-plots of pungent odour liking are shown in Figure 17, with 3 Principal Components (PC) explaining 44.35 % of the data variance. The graph PC1-PC2 (Figure 17a) shows that most of consumers liked HD sample and disliked SD sample. However, solutions of 0.06 µl/l and 0.23 µl/l 1P3O were grouped together with SD sample. Figure 17a also shows that most light (L1-L40) and moderate (M1-M40) chilli-users liked pungent odour of

dried chilli at low (0.61 g/l) and moderate (1.75 g/l) concentrations, respectively. To interpret the PCA results, a consumer liking pattern can be demonstrated. For example, the consumer M29 extremely disliked 0.06 µl/l 1P3O sample, it means that he also disliked SD. For PC1-PC3 (Figure 17b), most heavy chilli-users like pungent odour of dried chilli at the strong intensity (5.88 g/l) and they may also dislike SD samples extremely.

Table 35 Liking mean scores on pungent odour and hotness of different intensities among the three consumer groups

Sample concentration	Light chilli-users (n=40)	Moderate chilli-users (n=40)	Heavy chilli-users (n=40)
Pungent odour of dried chilli			
0.38 g/l	$5.48\pm1.74^{b,A}$	$5.40\pm1.81^{b,A}$	$5.58\pm1.52^{b,A}$
0.61 g/l	$6.52\pm1.24^{ab,A}$	$5.50\pm1.47^{ab,A}$	$5.60\pm1.66^{ab,A}$
1.75 g/l	$6.03\pm1.42^{ab,A}$	$6.32\pm1.61^{ab,A}$	$5.70\pm1.59^{ab,A}$
5.88 g/l	$5.90\pm1.30^{a,A}$	$5.95\pm1.74^{a,A}$	$6.58\pm1.03^{a,A}$
9.53 g/l	$5.28\pm1.74^{b,A}$	$5.68\pm1.27^{b,A}$	$5.95\pm1.57^{b,A}$
Pungent odour of 1P3O			
0.04 µl/l	$5.10\pm1.15^{b,A}$	$5.48\pm1.30^{b,A}$	$4.90\pm1.61^{b,A}$
0.06 µl/l	$6.33\pm1.12^{a,A}$	$5.52\pm1.58^{a,A}$	$5.27\pm1.57^{a,A}$
0.23 µl/l	$5.15\pm0.89^{a,A}$	$6.07\pm1.12^{a,A}$	$5.42\pm1.63^{a,A}$
1.34 µl/l	$4.85\pm1.03^{a,A}$	$5.62\pm1.64^{a,A}$	$6.25\pm1.37^{a,A}$
2.17 µl/l	$4.68\pm1.29^{b,A}$	$4.90\pm1.60^{b,A}$	$5.60\pm1.26^{b,A}$
Hotness of dried chilli			
0.36 g/l	$5.35\pm1.58^{bc,A}$	$5.85\pm1.59^{bc,A}$	$4.85\pm1.96^{bc,A}$
0.58 g/l	$6.40\pm1.34^{a,A}$	$5.15\pm1.79^{a,A}$	$5.90\pm1.57^{a,A}$
2.23 g/l	$5.82\pm1.62^{ab,A}$	$6.10\pm1.66^{ab,A}$	$5.18\pm1.65^{ab,A}$
7.19 g/l	$5.60\pm1.68^{ab,A}$	$5.10\pm1.79^{ab,A}$	$6.30\pm1.24^{ab,A}$
11.65 g/l	$4.75\pm1.97^{c,A}$	$4.75\pm1.79^{c,A}$	$5.67\pm1.56^{c,A}$
Hotness of capsaicin			
0.59 mg/l	$5.20\pm1.20^{b,A}$	$5.28\pm1.99^{b,A}$	$4.98\pm1.64^{b,A}$
0.96 mg/l	$6.38\pm1.13^{a,A}$	$5.88\pm1.16^{a,A}$	$5.10\pm1.71^{a,A}$
2.36 mg/l	$5.65\pm1.49^{a,A}$	$6.13\pm1.36^{a,A}$	$5.25\pm1.69^{a,A}$
12.79 mg/l	$4.88\pm1.49^{ab,A}$	$5.17\pm0.98^{ab,A}$	$6.25\pm1.10^{ab,A}$
20.72 mg/l	$4.55\pm1.68^{b,A}$	$5.15\pm1.72^{b,A}$	$5.57\pm1.50^{b,A}$

Note: Different small superscripts within sample concentration of each attribute refer to the significant difference ($P\leq0.05$).
Different capital superscripts among consumer groups in each attribute refer to the significant difference ($P\leq0.05$).

The hotness liking patterns are not conclusive from overall when considering PCA results shown in Figure 18. However, it is worth looking at the outstanding samples which contributed to major data variation in the PCs1 and 2 (Figure 18a). The solutions of chilli 2.23 g/l and 0.36 g/l and capsaicin 20.72 mg/l were clearly at the boundary of liking. Combining with the ANOVA results (Tables 32 and 35), it can be interpreted that the consumers generally liked hotness of middle dried chilli (2.23 g/l) and low level capsaicin (0.96 mg/l) concentrations (Figure 18b). Both dried chilli and capsaicin concentrations of capsaicin were most liked by moderated and light chilli-users ($P \leq 0.05$) (Table 35).

Some details of individual liking patterns are shown in Figure 18a. For example, consumer M26 extremely disliked hotness intensities of SD and HD but he/ she liked very strong hotness of capsaicin (20.72 mg/l). Similarly, the consumer H21 disliked hotness of dried chilli, but he/ she extremely liked very strong hotness of dried chilli (11.65 g/l). These notions bring further discussion of influences of flavours on hotness perception and liking in natural food products.

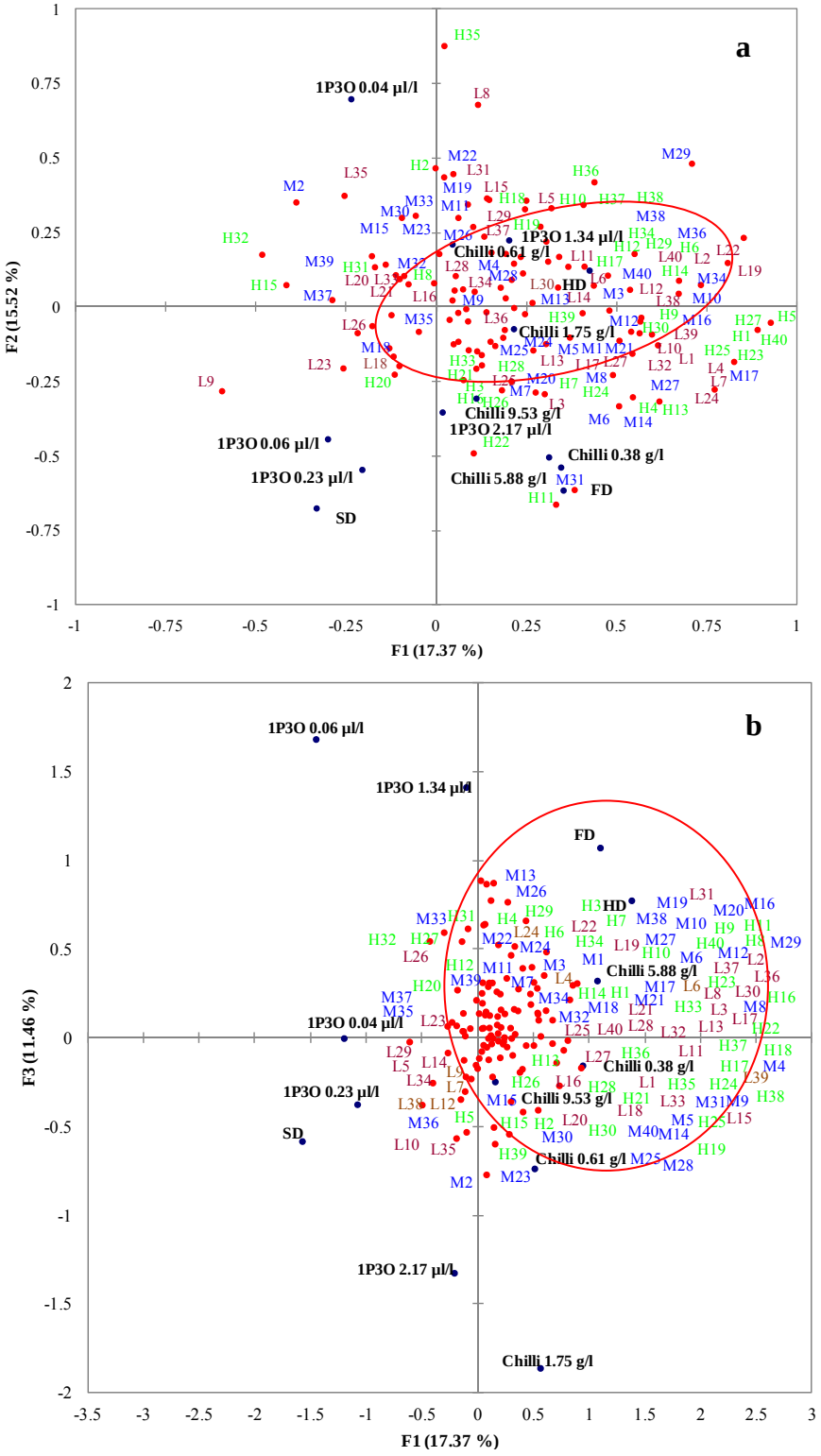


Figure 17 PCA bi-plot of pungent odour liking by chilli consumers (n=120); PC1-PC2 (a), PC1-PC3 (b)

Note: L1-L40 = Light chilli-users, M1-M40 = moderate chilli users and H1-H40 = Heavy chilli-users

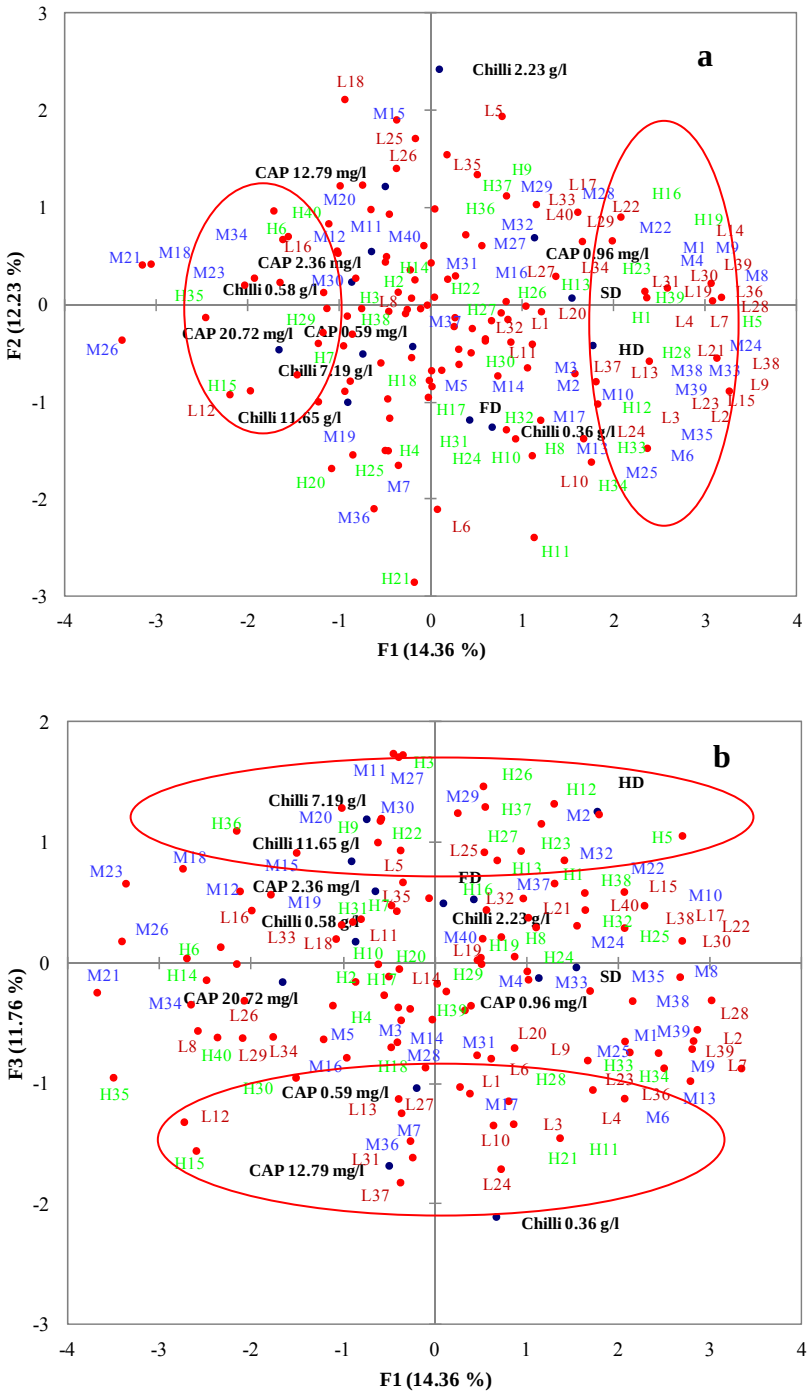


Figure 18 PCA bi-plots of hotness liking by chilli consumers (n=120); PC1-PC2 (a), PC1-PC3 (b)

Note: L1-L40 = Light chilli-users, M1-M40 = moderate chilli users and H1-H40 = Heavy chilli-users

When each group of chilli consumers tasted the same set of sample concentrations, the liking results confirm that the levels of hotness and pungent odour in the most liked samples were matched with the individual group thresholds. The heavy chilli-users who frequently consume chilli-containing foods, like high intensity of pungent odour and hotness. This might suggest that the samples of low to moderate hotness/pungent odour intense would have no affective response to this group. In addition, the consumers like the hotness/pungent odour as long as the level of hotness/pungent odour intensity is perceived as high enough to them (Rozin and Schiller, 1980). By analogy, Moskowitz *et al.* (1976) who studied consumer liking on bitter taste from quinine and found that consumers who were naive to quinine rated neither like nor disliked on the samples of low to moderately intense quinine solutions. They then disliked the solutions with more concentration of the substance.

The most liked hotness levels of dried chilli samples by light, moderate and heavy chilli users are fit well with the three hotness categories classified in SHU scale as 'mild', 'medium' and 'very hot'. The light chilli users liked hotness intensity of 15,360 SHU dried chilli, whist moderate and heavy chilli users liked dried chilli with hotness of 37,760 and 204,640 SHU, respectively. These liked hotness levels are in similar ranges of mild (0-35,000 SHU), medium (35,000-70,000 SHU) and very hot (70,000-16,000,000 SHU) chilli hotness classified by Thai Department of Agriculture (2013). The hotness levels that reported by the present research are also supported by the hotness levels of chilli classified by Nwokem *et. al*. (2010). The ranges were reported in three hotness levels with mild (0-25,000 SHU which had subunits of non-hotness (0-700 SHU), mildly hotness (700-3,000 SHU) and moderately hotness (3,000-25,000 SHU)), medium (25,000-70,000 SHU) and very hot (more than 80,000 SHU).

The labels of hotness level in chilli-contained products, however, may not be based on the criteria specified above. For example, the well-known commercial chilli-sauce 'Sriracha' has its own labels on hotness classification. To compare the hotness level of the commercial products, the percentages of chilli in the sauce ingredients of each category were calculated into SHU (Edge, 2009). It was reported that the 'mild' label contained hotness between 90-930 SHU, the 'medium' label did between 1,150-2,750 SHU and the 'very hot' label did between 3,000-30,000 SHU.

From this example of commercial chilli products, the classification of three hotness levels (mild, medium and very hot) is not similar to the classification of this research and other references as mentioned previously. They were rather low in SHU of all three hotness levels in this product. This is possible that the chilli sauce is sold in the US and elsewhere, thus its hotness levels may be classified according to the perceived threshold of target consumers.

5.5 Conclusion

Three groups of chilli-users agreed to like hot air dried chilli most for its hotness, pungent odour and flavour. However, the preferred levels of hotness and pungent odour intensities of the dried chilli and standard samples were different. The most preferred sample concentration of each consumer group was of similar concentration level to the group's threshold. Light chilli-users liked hotness and pungent odour of dried chilli at concentrations of 0.58 and 0.61 g/l (mild level). Moderate chilli-users preferred moderate level of dried chilli hotness and pungent odour with concentrations of 2.23 and 1.75 g/l, respectively. The heavy chilli-users liked strong level of dried chilli hotness and pungent odour most at the concentrations of 7.19 and 5.88 g/l. This study proposes that the amount of chilli content in capsaicin food products affects consumer hotness and pungent odour liking, hence overall product acceptance can be different by chilli-user groups. In order to possibly increase consumer acceptance, the commercial spicy food products may attempt to enhance the chilli content up to hotness recognition thresholds for individual groups of consumers.

CHAPTER 6

SUMMARY AND FUTURE WORK

6.1 Summary

In this research, three current drying methods of chilli (freeze, hot air and sun drying) were compared based on their products' physical and chemical qualities, including capsaicin content and volatile flavour compounds. Freeze drying method produced a bright-red colour dried chilli and presented higher quantity of pungent odour compound (1-penten-3-one; 1P3O) and ascorbic acid content in its product compared with the other two drying methods. These were affected by low temperature and limited exposure to oxygen during the process. All three drying methods did not show significant effects on the level of capsaicin found in the dried chilli samples.

Even though both capsaicin and 1P3O compounds were investigated in this research and elsewhere, dihydrocapsaicin might also be another potential compound contributing to hotness perception. Likewise, β-caryophyllene (spicy odour), 2-octanol (spicy odour) and 3-chloro-benzaldehyde (pungent odour) could potentially be compounds producing pungent odour similar to 1P3O.

This research has established hotness and pungent odour profiles of dried Chee fah chilli that were perceived by sensory panel. The profiles provide typical features of dried chilli which can be related to consumer acceptance. The three dried chilli samples were discriminated according to their hotness and pungent odour-related profiles. The freeze dried chilli sample had the highest intensities in most of hotness-related attributes when compared with the two others. The hot air dried chilli presented similar intensities of pungent odour-related attributes to the freeze dried chilli. The sun dried chilli contained the least of fresh chilli odour, sting-pungent odour, oral sting and warm in mouth.

The research finding had clearly shown that the threshold levels determined by ASTM E1432 were similar to the thresholds levels determined by ASTM E679. The recognition thresholds derived from the three consumer groups are reliable. This conclusion is specially drawn on light chilli-users who consumed chilli

in small quantity and with the lowest frequency, that they were most sensitive to hotness and pungent odour stimuli. They had low level of thresholds and generally liked mild samples (approximately 0.96 mg/l capsaicin and 0.58 g/l dried chilli). The recognition hotness threshold of Thai light chilli-users was higher than hotness threshold reported in European consumers (0.08 mg/l capsaicin) and Japanese consumers (0.70 mg/l capsaicin) but of similar threshold range with Turkish consumers (1.53 mg/l capsaicin). It is to note that the determined recognition threshold derived from the present study may not reflect the threshold of Thai population because the specific age group of 18-35 years old were investigated.

Despite the bright red colour and intense hotness were presented in freeze dried chilli, the three groups of Thai consumers had given the highest liking scores, on hotness, pungent odour and flavour in the hot air sample. This finding was discussed in relation to 'familiarity' of the sample among Thai consumers. Moreover, it was found that the consumers liked hotness and pungent odour at the same intensity level as their recognition threshold ranges. For example, most of the heavy chilli-users liked the highest intensities of hotness and pungent odour stimuli (7.19 and 5.88 g/l dried chilli, respectively). The threshold levels and the liking scores were well correlated with the consumers' frequency of consumption and amount of chilli regularly consumed. The threshold of target group is inevitably required in order to apply chilli in commercial products. The claims of hotness levels on the product labels hence are varied and may not be similar heat unit standard (i.e. SHU) in chilli itself. In the case of a commercial chilli sauce (i.e. Sriracha chilli-sauce), the labelled product as 'very hot' actually contains hotness level of 3,000-25,000 SHU which is much lower that the SHU of the same hotness category specified in this research for raw material. The research findings give a guideline for applying chilli in food products in order to gain consumer preferences from different segments.

6.2 Future work

Since the research samples are based on standard solutions and a singular ingredient (dried chilli), it could be more beneficial to food industry if the food matrix model is applied where there would be interactions occurring between hotness and other senses.

1. Further studies may focus on the influences of hotness contributed by the two sensory attributes (hotness and pungent odour) on taste perceptions.

2. Other hotness compounds (e.g. dihydrocapsaicin) and other pungent odour compounds (e.g. β-caryophyllene, 2-octanol and 3-chloro-benzaldehyde) may also be investigated regarding their potential effects on hotness perception.

3. The chilli consumption pattern has indicated consumer threshold of hotness. When chilli, capsaicin or 1P3O are applied in formulating a food product, there would be influences from the product's ingredients on consumer overall perception and liking. The results might not be as straightforward as what has been found from the single stimulus used in this research. The differences of perception and liking might not be very apparent among the three user groups.

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Appendix

Appendix 1

Raw Material and Experimental Samples

Figure 1 Fresh Chee fah chilli

(a) (b) (c)

Figure 2 Chee fah chilli dried by sun (a), hot air (b) and freeze (c)-drying methods

(a) (b) (c)

Figure 3 Ground samples of Chee fah chilli dried by sun (a), hot air (b) and freeze (c)-drying methods

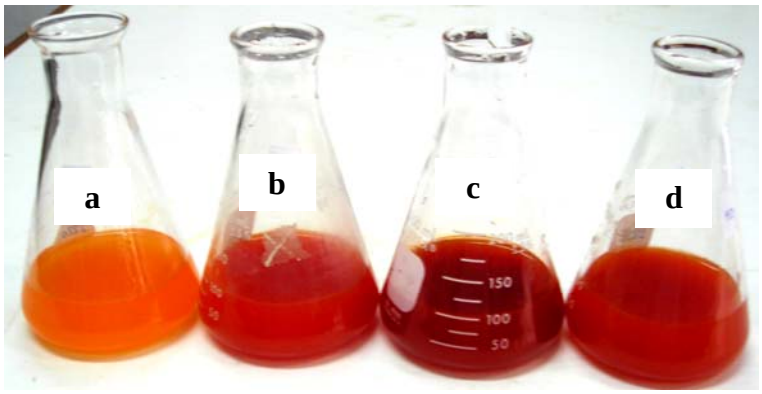


Figure 4 Solution of Chee fah chilli from fresh chilli (a), sun (b), hot air (c) and freeze (d)-drying methods

Appendix 2

Chromatogram of Volatile Flavour Compounds Identified by LLE and SPME/GC-MS and Standard Curve of Ascorbic Acid

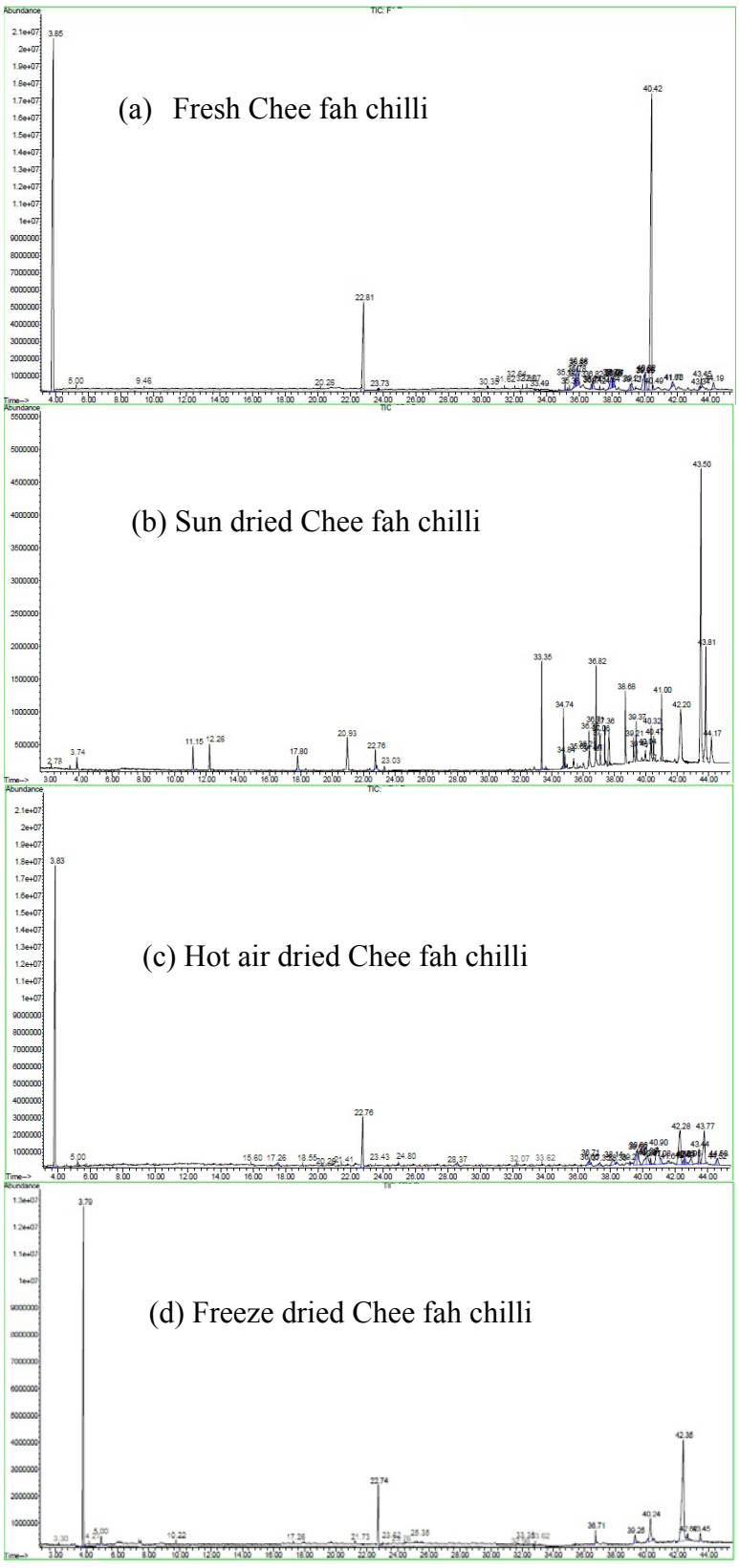


Figure 5 Chromatograms of volatile flavour compounds in fresh and dried Chee fah chilli extracted and analysed by LLE/GC-MS

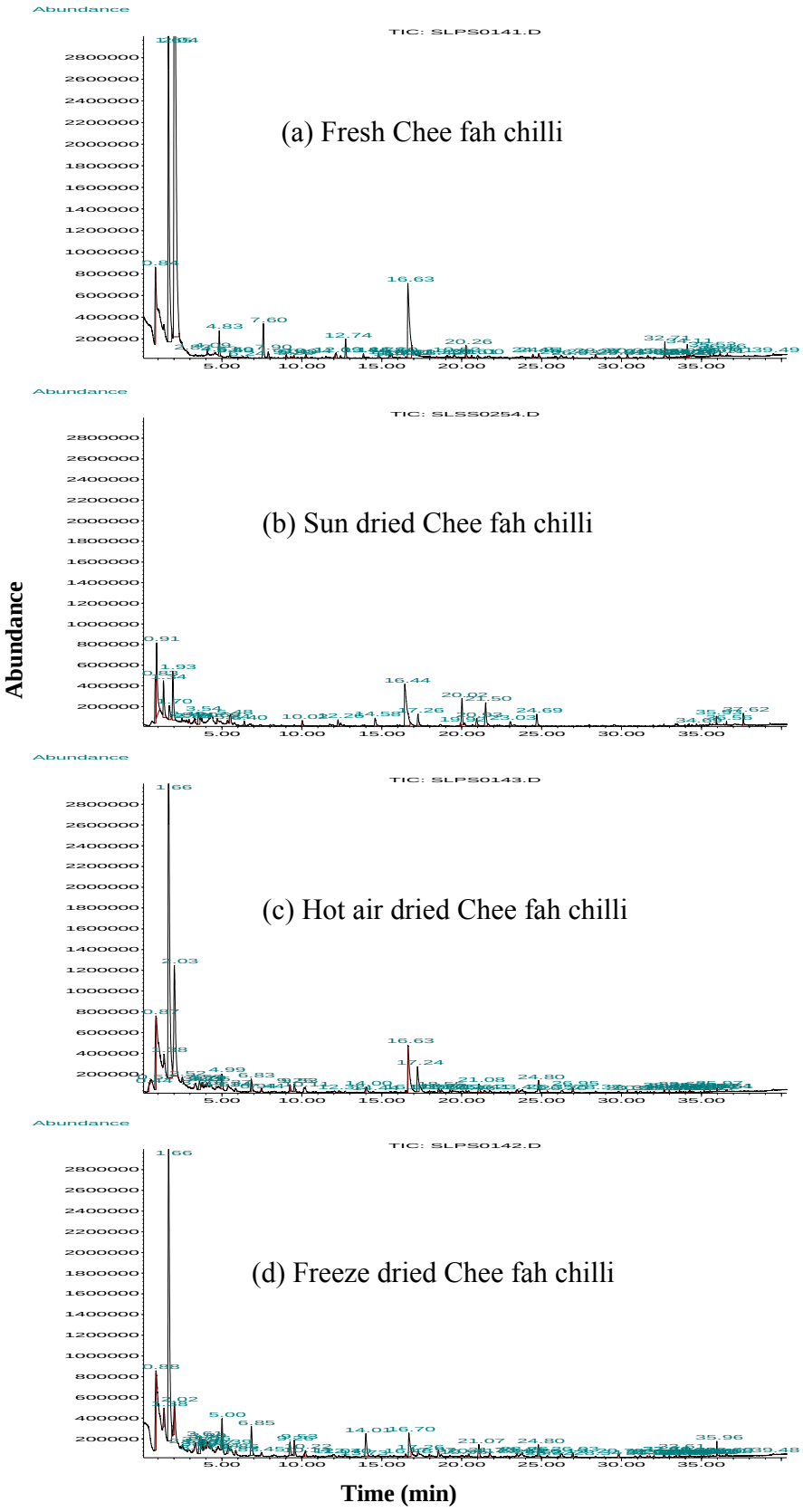


Figure 6 Chromatograms of volatile flavour compounds in fresh and dried Chee fah chilli extracted and analysed by SPME/GC-MS

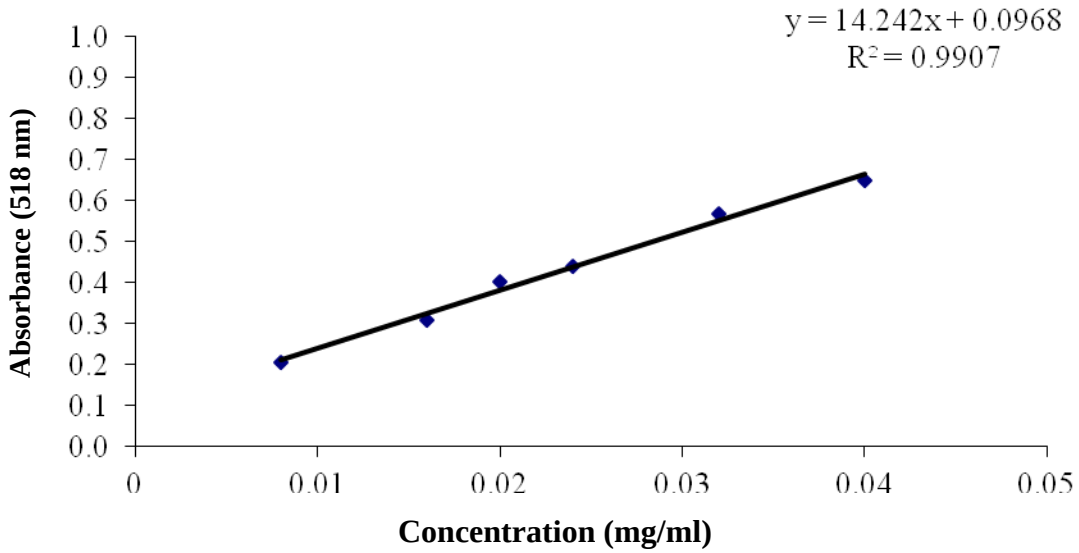


Figure 7 Calibration curve for determination of ascorbic acid

Table 1 Concentration of ascorbic acid standards and absorbance (518 nm) value

Concentration (mg/ml)	Absorbance of dilution	Absorbance for standard curve
0.00	0.589	-
0.20	0.483	0.106
0.40	0.382	0.207
0.60	0.287	0.302
0.80	0.148	0.441
1.00	0.082	0.507

Note: Absorbance for standard curve was calculated by absorbance of control (0.00 mg/l ascorbic acid) – absorbance of sample (Sroka and Cisowski, 2005).

Appendix 3

Session of Panel Training for Descriptive Analysis

Appendix 3.1 Session of screening panellsist

Table 2 Reference samples for screening panellists

Sample codes	Attributes	Reference samples	Concentrations
Part 1			
347	FO	Fresh chilli	2.5 g
519	Hot air dried chilli odour	Hot air dried chilli	2.5 g
156	Sun dried chilli odour	Sun dried chilli	2.5 g
472	Freeze dried chilli odour	Freeze dried chilli	2.5 g
798	Chilli pungent odour	1-pentene-3-one solution	0.2 µl/l (10 ml)
178	Galangal odour	Galangal	2.5 g
863	Pepper odour	Pepper	2.5 g
Part 2			
Sample set 1			0.1mg/l
248	Mild hotness	capsaicin solution	0.3 mg/l
372			0.4 mg/l
352			0.8 mg/l
273			
Sample set 2			
537			1 mg/l
491	Medium hotness	capsaicin solution	2 mg/l
579			4 mg/l
468			6 mg/l
Sample set 3			
537			1 mg/l
491	Very hotness	capsaicin solution	2 mg/l
579			4 mg/l
468			6 mg/l
Sample set 4			
768			0.2 µl/l
462	Pungent odour	1P3O solution	0.4 µl/l
653			0.8 µl/l
421			1.5 µl/l
Part 3			
395	Hotness	Fresh chilli solution	2.5 g/l
114		Hot aired dried chilli solution	2.5 g/l
Part 4			
813	Pungent odour	Fresh chilli solution	2.5 g/l
948		Hot aired dried chilli solution	2.5 g/l

Appendix 3.2 Session of developing lexicon and training panellsits

Table 3 Reference samples for developing lexicon at hour 3rd-4th

Sample codes	Reference samples
539	2.5 g ground fresh chilli
692	2.5 g ground hot air dried chilli
713	2.5 g ground hot air dried chilli
861	2.5 g ground freeze dried chilli
117	2.5 g ground fresh galangal
216	2.5 g ground fresh ginger
432	2.5 g ground fresh cumin
715	2.5 g ground dried black pepper

Table 4 Reference samples for developing lexicon at hour 5th-6th

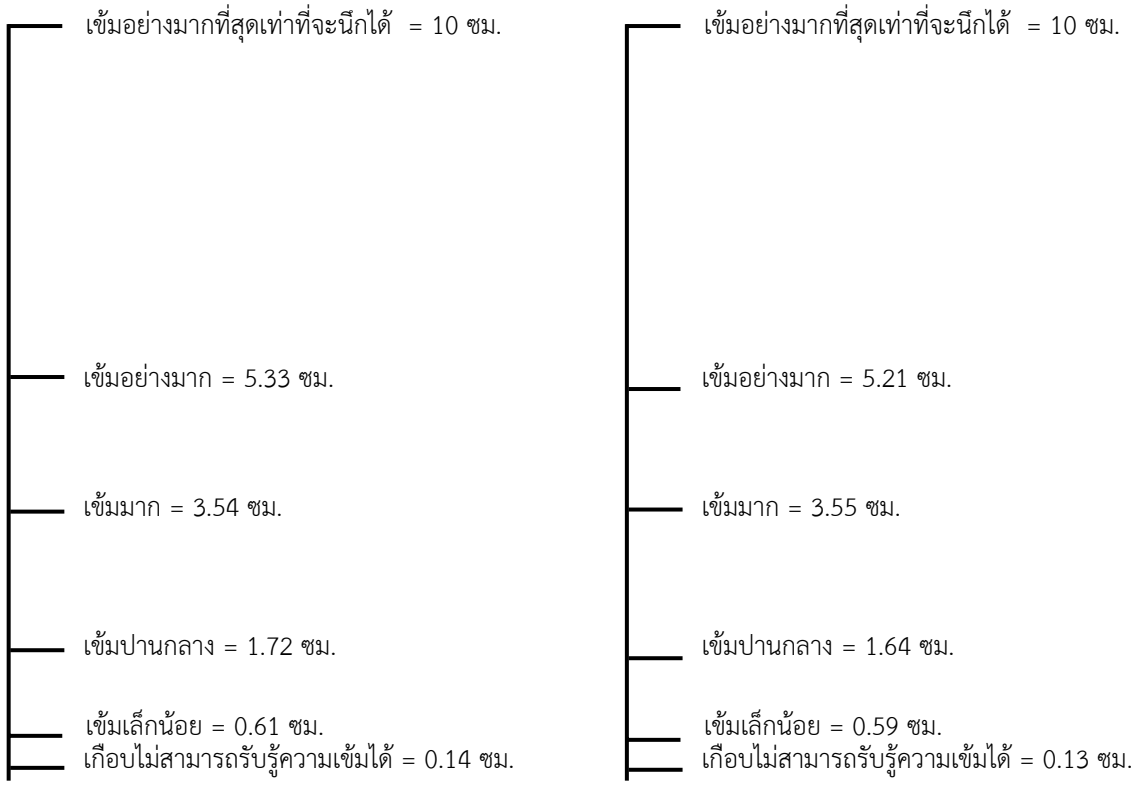
Sample codes	Reference samples
174	2.5 g/l solution of fresh chilli
897	2.5 g/l solution of fresh galangal
562	2.5 g/l solution of fresh ginger
641	2.5 g/l solution of fresh cumin
283	2.5 g/l solution of dried black pepper

Table 5 Reference samples for developing lexicon at hour 7th- 8th

Sample codes	Reference samples
283	2.5 g/l solution of fresh chilli
862	2.5 g/l solution of sun dried chilli
746	2.5 g/l solution of air dried chilli
835	2.5 g/l solution of freeze dried chilli
654	0.23 µl/l solution of 1-penten-3-one
807	2.36 mg/l solution of capsaicin

Table 6 Verbal descriptors for LMS scale at hour 9th

Verbal descriptors	Final consensuses of descriptor in Thai and the ranking in descending order
1) Strongest imaginable	1). เข้มอย่างมากที่สุดเท่าที่จะนึกได้
2) Very strong	2). เข้มรุนแรงมาก
3) Strong	3). เข้มมาก
4) Moderate	4) เข้มปานกลาง
5) Weak	5). เข้มเล็กน้อย
6) Barely detectable	6). เกือบไม่สามารถรับรู้ความเข้มได้



(a) (b)

Figure 8 A 10 cm vertical Labelled Magnitude Scale (LMS) with the six verbal anchors (a) and new LMS scale (b) derived from the panellist group at hour 10^{th}

Note: Scale (b) was used in training (Appendix 4.4, Section 4.4.1.1) and sample evaluation (Appendix 4.4, Section 4.4.2)

Table 7 Samples for LMS scaling at hour 11^{th}- 12^{th}

Sensory attributes	Codes	Reference samples
DRC	R1	0.45 g/l fresh chilli
	R2	tomato sauce (Roza brand)
BO	R3	pure water
	R4	2.5 g ground roast chilli (80^oC, 10 min)
FO	R5	vinegar
	R6	2.5 g ground fresh chilli

Note: All reference samples (R1-R6) were presented together with experimental samples, namely 2.5 g of ground fresh chilli, sun, hot air and freeze-dried chilli samples, 2.5 g/l solution of fresh chilli, sun, hot air and freeze-dried chilli.

Table 8 Samples for LMS scaling at hour 13^{th}-14^{th} and 15^{th}-16^{th}

at hour 13^{th} and 14^{th}			at hour 15^{th} and 16^{th}		
Sensory attributes	**Codes**	**Reference samples**	**Sensory attributes**	**Codes**	**Reference samples**
RNO	R7	pure water	OB	R15	pure water
	R8	2.04 µl/l 1P3O		R16	15 mg/l capsaicin
SPO	R9	pure water	OBS	R17	pure water
	R10	2.04 µl/l 1P3O		R18	15 mg/l capsaicin
WM	R11	pure water	OS	R19	pure water
	R12	15 mg/l capsaicin		R20	15 mg/l capsaicin
WMS	R13	pure water	OSS	R21	pure water
	R14	15 mg/l capsaicin		R22	15 mg/l capsaicin
			TN	R23	pure water
				R24	15 mg/l capsaicin

Note: All reference samples (R7-R24) were presented together with experimental samples, namely 0.20 and 0.40 µl/l solution of 1-penten-3-one (1P3O), 2.00 and 4.00 mg/l solution of capsaicin, 2.5 g of ground fresh chilli and 2.5 g/l solution of fresh chilli.

Table 9 Samples for LMS scaling at hour 17th-18th and 19th-20th

at hour 13th and 14th			at hour 15th and 16th		
Sensory attributes	**Codes**	**Reference samples**	**Sensory attributes**	**Codes**	**Reference samples**
RNO	R7	pure water	OB	R15	pure water
	R8	2.04 µl/l 1P3O		R16	15 mg/l capsaicin
SPO	R9	pure water	OBS	R17	pure water
	R10	2.04 µl/l 1P3O		R18	15 mg/l capsaicin
WM	R11	pure water	OS	R19	pure water
	R12	15 mg/l capsaicin		R20	15 mg/l capsaicin
WMS	R13	pure water	OSS	R21	pure water
	R14	15 mg/l capsaicin		R22	15 mg/l capsaicin
			TN	R23	pure water
				R24	15 mg/l capsaicin

Note: All reference samples (R7-R24) were presented together with experimental samples, namely 2.5 g of ground sun, hot air and freeze-dried chilli samples, 2.5 g/l solution of sun, hot air and freeze-dried chilli.

Table 10 Samples for ULS scaling at hour 21st-22nd

Sensory attributes	Codes	Reference samples
RNO	R7	pure water
	R8	2.04 µl/l 1P3O
SPO	R9	pure water
	R10	2.04 µl/l 1P3O
WM	R11	pure water
	R12	15 mg/l capsaicin
WMS	R13	pure water
	R14	15 mg/l capsaicin

Note: All reference samples (R7-R14) were presented together with experimental samples, namely 0.20 and 0.40 µl/l solution of 1-penten-3-one (1P3O), 2.00 and 4.00 mg/l solution of capsaicin, 2.5 g of ground fresh chilli and 2.5 g/l solution of fresh chilli.

Table 11 Samples for ULS scaling at hour 23rd-24th and 25th-26th

at hour 23rd-24th			at hour 25th-26th		
Sensory attributes	**Codes**	**Reference samples**	**Sensory attributes**	**Codes**	**Reference samples**
RNO	R7	pure water	OB	R13	pure water
	R8	2.04 µl/l 1P3O		R14	15 mg/l capsaicin
SPO	R9	pure water	OS	R15	pure water
	R10	2.04 µl/l 1P3O		R16	15 mg/l capsaicin
WM	R11	pure water	TN	R17	pure water
	R12	15 mg/l capsaicin		R18	15 mg/l capsaicin

Note: All reference samples (R7-R18) were presented together with experimental samples, namely 2.5 g of ground sun, hot air and freeze-dried chilli samples, 2.5 g/l solution of sun, hot air and freeze-dried chilli.

Table 12 Samples for testing performance of panellist at hour 27th-32nd (The1st test)

Sensory attributes	Codes	Reference samples
DRC	R1	0.45 g/l fresh chilli
	R2	tomato sauce (Roza brand)
BO	R3	pure water
	R4	2.5 g ground roast chilli (80°C, 10 min)
FO	R5	vinegar
	R6	2.5 g ground fresh chilli
RNO	R7	pure water
	R8	2.04 µl/l 1P3O
SPO	R9	pure water
	R10	2.04 µl/l 1P3O
WM	R11	pure water
	R12	15 mg/l capsaicin
WMS	R13	pure water
	R14	15 mg/l capsaicin
OB	R15	pure water
	R16	15 mg/l capsaicin
OBS	R17	pure water
	R18	15 mg/l capsaicin
OS	R19	pure water
	R20	15 mg/l capsaicin
OSS	R21	pure water
	R22	15 mg/l capsaicin
TN	R23	pure water
	R24	15 mg/l capsaicin

Note: All reference samples (R1-R24) were presented together with experimental samples, namely 0.40, 0.80 and 1.5 µl/l solution of 1penten-3-one (1P3O), 2.00 and 6.00 mg/l solution of capsaicin.

Table 13 Samples for testing performance of panellist at hour 33rd-34th and hour 35th-36th

at hour 33rd and 34th			at hour 35th and 36th		
Sensory attributes	**Codes**	**Reference samples**	**Sensory attributes**	**Codes**	**Reference samples**
DRC	R1	0.45 g/l fresh chilli	WM	R11	pure water
	R2	tomato sauce (Roza brand)		R12	15 mg/l capsaicin
BO	R3	pure water	WMS	R13	pure water
	R4	2.5 g ground roast chilli		R14	15 mg/l capsaicin
FO	R5	(80°C, 10 min)	OB	R15	pure water
	R6	vinegar		R16	15 mg/l capsaicin
RNO	R7	2.5 g ground fresh chilli	OBS	R17	pure water
	R8			R18	15 mg/l capsaicin
SPO	R9	pure water	OS	R19	pure water
	R10	2.04 µl/l 1P3O		R20	15 mg/l capsaicin
		pure water	OSS	R21	pure water
		2.04 µl/l 1P3O		R22	15 mg/l capsaicin
			TN	R23	pure water
				R24	15 mg/l capsaicin

Note: All reference samples (R1-R24) were presented together with experimental samples, namely 2.5 g of ground sun, hot air and freeze-dried chilli samples, 2.5 g/l solution of sun, hot air and freeze-dried chilli.

Table 14 Samples for testing performance of panellist at hour 37th-42nd (The 2nd test)

Sensory attributes	Codes	Reference samples
DRC	R1	0.45 g/l fresh chilli
	R2	tomato sauce (Roza brand)
BO	R3	pure water
	R4	2.5 g ground roast chilli (80°C, 10 min)
FO	R5	vinegar
	R6	2.5 g ground fresh chilli
RNO	R7	pure water
	R8	2.04 µl/l 1P3O
SPO	R9	pure water
	R10	2.04 µl/l 1P3O
WM	R11	pure water
	R12	15 mg/l capsaicin
WMS	R13	pure water
	R14	15 mg/l capsaicin
OB	R15	pure water
	R16	15 mg/l capsaicin
OBS	R17	pure water
	R18	15 mg/l capsaicin
OS	R19	pure water
	R20	15 mg/l capsaicin
OSS	R21	pure water
	R22	15 mg/l capsaicin
TN	R23	pure water
	R24	15 mg/l capsaicin

Note: All reference samples (R1-R24) were presented together with experimental samples, namely 0.87 g/l, 2.23 g/l and 5.71 g/l solution of hot air dried chilli.

Table 15 Sensory lexicon and test protocol (in Thai)

ลักษณะทางประสาทสัมผัส	นิยาม	ตัวอย่างอ้างอิง		วิธีการประเมินตัวอย่าง	
		ความเข้มข้นต่ำ	ความเข้มข้นสูง	ความเข้มของลักษณะที่สัมพันธ์กับความเผ็ดและกลิ่นฉุน	ความเข้มของลักษณะที่สัมพันธ์กับกลิ่นรสฉุนและเผ็ด
1.สีแดงคล้ำ	ลักษณะสีแดงที่มองเห็นด้วยสายตา	พริกสดความเข้มข้น 0.45 กรัม/ลิตร	ซอสมะเขือเทศตราโรซ่า	ประเมินสีแดงคล้ำของตัวอย่างโดยการดู	ประเมินสีแดงคล้ำของตัวอย่างโดยการดู
2.กลิ่นพริกไหม้	กลิ่นไหม้เหมือนพริกที่ผ่านการให้ความร้อนโดยการอบที่รับรู้ได้ขณะดมตัวอย่าง	น้ำสะอาด	พริกอบแห้ง (80 องศาเซลเซียส, 10 นาที) 2.5 กรัม	ประเมินกลิ่นไหม้ของพริกโดยการสูดดม ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที แล้วประเมิน	ประเมินกลิ่นพริกไหม้โดยการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที เคลื่อนไหวภายในปากให้น้อยที่สุด คายตัวอย่าง จากนั้นรอ 30 วินาที แล้วประเมิน
3. กลิ่นพริกสด	กลิ่นฉุนเหม็นเขียว เหมือนพริกสดและไม่มีกลิ่นหมักเปรี้ยวที่รับรู้ได้ขณะดมตัวอย่าง	น้ำส้มสายชู	2.5 กรัม พริกสดบด	ประเมินกลิ่นพริกสดของพริกโดยการสูดดม ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที แล้วประเมิน	ประเมินกลิ่นพริกสดโดยการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที เคลื่อนไหวภายในปากให้น้อยที่สุด คายตัวอย่าง จากนั้นรอ 30 วินาที แล้วประเมิน
4.กลิ่นฉุนขึ้นจมูก	กลิ่นฉุนพริกจนระคายเคืองขึ้นจมูกขณะดมตัวอย่าง	น้ำสะอาด	สารมาตรฐาน 1P3O ความเข้มข้น 2.04 ไมโครลิตร/ลิตร	ประเมินความรู้สึกฉุนขึ้นจมูกโดยการการสูดดม ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที แล้วประเมิน	ประเมินความรู้สึกฉุนขึ้นจมูกโดยการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที เคลื่อนไหวภายในปากน้อยที่สุด คายตัวอย่าง จากนั้นรอ 30 วินาที แล้วประเมิน
5.กลิ่นฉุนแสบจมูก	กลิ่นฉุนพริกจนรู้สึกแสบจมูกขณะดมตัวอย่าง	น้ำสะอาด	สารมาตรฐาน 1P3O ความเข้มข้น 2.04 ไมโครลิตร/ลิตร	ประเมินความรู้สึกฉุนแสบจมูกโดยการการสูดดม ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที แล้วประเมิน	ประเมินความรู้สึกฉุนแสบจมูกโดยการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที เคลื่อนไหวภายในปากให้น้อยที่สุด คายตัวอย่าง จากนั้นรอ 30 วินาที แล้วประเมิน
6.ความรู้สึกอุ่นในปาก	ความรู้สึกอุ่นในปากขณะที่ตัวอย่างอยู่ในปาก	น้ำสะอาด	สารมาตรฐาน capsaicin ความเข้มข้น 15 มิลลิกรัม/ลิตร	ประเมินความรู้สึกอุ่นภายในปากโดยใช้ที่หนีบจมูก และการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที เคลื่อนไหวภายในให้ปากน้อยที่สุด ประเมิน	ประเมินความรู้สึกอุ่นภายในปากโดยการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที เคลื่อนไหวภายในให้ปากน้อยที่สุด ประเมิน

Table 15 Continued

ลักษณะทางประสาทสัมผัส	นิยาม	ตัวอย่างอ้างอิง		วิธีการประเมินตัวอย่าง	
		ความเข้มข้นต่ำ	ความเข้มข้นต่ำ	ความเข้มของลักษณะที่สัมพันธ์กับความเผ็ดและกลิ่นฉุน	ความเข้มของลักษณะที่สัมพันธ์กับกลิ่นรสฉุนและเผ็ด
7. ความรู้สึกอุ่นในปากหลังคาย	ความรู้สึกอุ่นในปากหลังจากการคายตัวอย่างทิ้ง	น้ำสะอาด	สารมาตรฐาน capsaicin ความเข้มข้น 15 มิลลิกรัม/ลิตร	ประเมินความรู้สึกอุ่นภายในปากโดยใช้ที่หนีบจมูก และอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที โดยเคลื่อนไหวภายในปากให้น้อยที่สุด จากนั้นคายตัวอย่าง รอ 30 วินาที แล้วประเมิน	ประเมินความรู้สึกอุ่นภายในปากโดยการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที เคลื่อนไหวภายในปากให้น้อยที่สุด จากนั้นคายตัวอย่าง รอ 30 วินาที แล้วประเมิน
8. ความเผ็ดร้อนในปาก	ความรู้สึกเผ็ดร้อนในปากเหมือนปากไหม้พองขณะที่ตัวอย่างอยู่ในปาก	น้ำสะอาด	สารมาตรฐาน capsaicin ความเข้มข้น 15 มิลลิกรัม/ลิตร	ประเมินความรู้สึกเผ็ดร้อนภายในปากโดยใช้ที่หนีบจมูก และการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที โดยเคลื่อนไหวภายในปากให้น้อยที่สุด แล้วประเมิน	ประเมินความรู้สึกเผ็ดร้อนภายในปากโดยการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที เคลื่อนไหวภายในปากให้น้อยที่สุด แล้วประเมิน
9. ความเผ็ดร้อนในปากหลังคาย	ความรู้สึกเผ็ดร้อนในปากเหมือนปากไหม้พองหลังจากการคายตัวอย่างทิ้ง	น้ำสะอาด	สารมาตรฐาน capsaicin ความเข้มข้น 15 มิลลิกรัม/ลิตร	ประเมินความรู้สึกเผ็ดร้อนภายในปากโดยใช้ที่หนีบจมูก และอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที โดยเคลื่อนไหวภายในปากน้อยที่สุด จากนั้นคายตัวอย่าง รอ 30 วินาที แล้วประเมิน	ประเมินความรู้สึกเผ็ดร้อนภายในปากโดยการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที เคลื่อนไหวภายในปากให้น้อยที่สุด จากนั้นคายตัวอย่าง รอ 30 วินาที แล้วประเมิน
10. ความเผ็ดแสบในปาก	ความรู้สึกเจ็บแสบในปากเหมือนมีเข็มทั่วปาก ซึ่งเกิดจากความเผ็ดขณะที่ตัวอย่างอยู่ในปาก	น้ำสะอาด	สารมาตรฐาน capsaicin ความเข้มข้น 15 มิลลิกรัม/ลิตร	ประเมินความรู้สึกเผ็ดแสบภายในปากโดยใช้ที่หนีบจมูก และการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที โดยเคลื่อนไหวภายในปากให้น้อยที่สุด แล้วประเมิน	ประเมินความรู้สึกเผ็ดแสบภายในปากโดยการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที เคลื่อนไหวภายในให้ปากน้อยที่สุด แล้วประเมิน
11. ความเผ็ดแสบในปากหลังคาย	ความรู้สึกเจ็บแสบในปากเหมือนมีเข็มทั่วปาก ซึ่งเกิดจากความเผ็ดหลังจากการคายตัวอย่างทิ้ง	น้ำสะอาด	สารมาตรฐาน capsaicin ความเข้มข้น 15 มิลลิกรัม/ลิตร	ประเมินความรู้สึกเผ็ดแสบภายในปากโดยใช้ที่หนีบจมูก และอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที โดยเคลื่อนไหวภายในปากน้อยที่สุด จากนั้นคายตัวอย่าง รอ 30 วินาที แล้วประเมิน	ประเมินความรู้สึกเผ็ดแสบภายในปากโดยการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที เคลื่อนไหวภายในปากน้อยที่สุด จากนั้นคายตัวอย่าง รอ 30 วินาที แล้วประเมิน
12. ลิ้นชา	ความรู้สึกชาที่ลิ้นหลังจากคายตัวอย่างทิ้ง	น้ำสะอาด	สารมาตรฐาน capsaicin ความเข้มข้น 15 มิลลิกรัม/ลิตร	ประเมินความรู้สึกชาที่ลิ้นโดยใช้ที่หนีบจมูก และอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที โดยเคลื่อนไหวภายในปากให้น้อยที่สุด จากนั้นคายตัวอย่าง รอ 30 วินาที แล้วประเมิน	ประเมินความรู้สึกชาที่ลิ้นโดยการอมตัวอย่างไว้ในปากเป็นเวลา 15 วินาที เคลื่อนไหวภายในปากให้น้อยที่สุด จากนั้นคายตัวอย่าง รอ 30 วินาที แล้วประเมิน

Appendix 4

Questionnaires for Sensory Evaluation

Appendix 4.1 Consent form for sensory evaluation (in Thai)

ใบชี้แจงข้อมูลและการแสดงความยินยอมเข้าร่วมการประเมินทางประสาทสัมผัสของผลิตภัณฑ์อาหารเผ็ด

ผลิตภัณฑ์อาหารเผ็ดที่ท่านจะได้ทดสอบชิมในวันนี้ ผลิตจากวัตถุดิบพืชที่ให้ความเผ็ด เตรียมด้วยน้ำสะอาดที่ผ่านการให้ความร้อนที่อุณหภูมิ 100 องศาเซลเซียส ท่านจะได้ชิมตัวอย่าง ทีละตัวอย่าง โดยการดม และอม พร้อมกับกลั้วให้ทั่วปาก หากท่านมีข้อสงสัยใด ๆ สามารถสอบถามนักวิจัย (นางสาวนิจฉรา ทูลธรรม นักศึกษาปริญญาเอก สาขาวิชาวิทยาศาสตร์และเทคโนโลยีอาหาร ภายใต้การดูแลของ ดร. มุทิตา มีนุ่น อาจารย์ประจำภาควิชาเทคโนโลยีอาหาร คณะอุตสาหกรรมเกษตร มหาวิทยาลัยสงขลานครินทร์) การทดสอบชิมจะใช้เวลาประมาณ 1 ชั่วโมง ทั้งนี้ท่านสามารถยกเลิกการทดสอบได้ทุกขณะที่ท่านต้องการ

ข้าพเจ้าได้รับข้อมูลตามที่ต้องการและยินดีเข้าร่วมการทดสอบประเมินทางประสาทสัมผัสของผลิตภัณฑ์อาหารเผ็ด ณ คณะอุตสาหกรรมเกษตร มหาวิทยาลัยสงขลานครินทร์

ลงชื่อ .....

( )

วันที่.....

Appendix 4.2 Questionnaire of spicy food consumption and interpretation for screening participant in sensory descriptive analysis, threshold and consumer testing (in Thai)

Appendix 4.2.1 Questionnaire

ส่วนที่ 1 ข้อมูลส่วนตัว

1. ชื่อ และนามสกุล.....
   ที่อยู่ (ที่พักปัจจุบัน).....
   โทรศัพท์ที่ติดต่อได้สะดวก.....อาชีพ..... อายุ..... ปี
   เคยมีประสบการณ์การทดสอบชิมมาก่อนหรือไม่.....
   ท่านเคยสูบบุหรี่ หรือไม่
   ☐ เคยสูบ และปัจจุบัน ☐ ไม่สูบแล้ว ☐ ยังสูบอยู่ ☐ ไม่เคยสูบ
   ท่านสนใจเข้าร่วมการทดสอบชิมผลิตภัณฑ์อาหารเผ็ดหรือไม่
   ☐ สนใจ (ถ้าสนใจกรุณาตอบข้อ 2) ☐ ไม่สนใจ

*<u>**หมายเหตุ</u>*: มีค่าตอบแทนให้กับผู้เข้าร่วมการทดสอบ

2. เวลาว่างที่สะดวกในการมาฝึกฝนเป็นผู้ชิมถาวรอาทิตย์ละ 1-2 ชั่วโมง เป็นระยะเวลา 2-3 เดือน

วัน/เวลา	จันทร์	อังคาร	พุธ	พฤหัสบดี	ศุกร์	เสาร์	อาทิตย์
10.00-11.30	.....	.....	.....	.....	.....	.....	.....
14.00-15.30	.....	.....	.....	.....	.....	.....	.....
18.00-19.30	.....	.....	.....	.....	.....	.....	.....

เวลาว่างที่สะดวกช่วงอื่นๆ (โปรดระบุ).....

3. ท่านมีอาการ/โรคประจำตัวต่อไปนี้ หรือไม่
   ☐ ไม่เป็น ☐ เป็น คือ

.....3.1 โรคหัวใจ .....3.2 โรคไต .....3.3 โรคความดันโลหิตสูง
.....3.4 โรคกระเพาะ .....3.5 คออักเสบ .....3.6 แพ้อาหารเผ็ด

ส่วนที่ 2 ข้อมูลเกี่ยวกับการรับประทานผลิตภัณฑ์อาหารเผ็ด

คำชี้แจง “ผลิตภัณฑ์อาหารเผ็ด” หมายถึง ผลิตภัณฑ์ที่มีพริกเป็นส่วนผสมและให้ลักษณะเด่นด้านความเผ็ด และกลิ่นฉุน

1) ท่านบริโภคอาหารที่รู้สึกว่าเผ็ดโดยเฉลี่ย

☐ 1 ครั้ง ต่อปี หรือน้อยกว่า ☐ น้อยกว่า 1 ครั้ง ต่อเดือน ☐ 1-3 ครั้งต่อเดือน

☐ 1 ครั้งต่อสัปดาห์ ☐ 3-4 ครั้งต่อสัปดาห์ ☐ ทุกวัน ☐ มากกว่า 1 ครั้งต่อวัน

2) จากผลิตภัณฑ์อาหารเผ็ดต่อไปนี้ ท่านเคยรับประทานผลิตภัณฑ์ใดบ้าง และเติมพริกมากน้อยเพียงใด (โปรดระบุจำนวนพริก)

2.1 ส้มตำ ☐ ไม่ใส่พริก ☐ ใส่พริก จำนวน.....เม็ด

2.2 ก๋วยเตี๋ยว ☐ ไม่ใส่พริก ☐ ใส่พริก จำนวน.....ช้อนชา พริกน้ำส้ม.....ช้อนชา

2.3 ยำ ☐ ไม่ใส่พริก ☐ ใส่พริก จำนวน.....เม็ด

2.4 ต้มยำ ☐ ไม่ใส่พริก ☐ ใส่พริก จำนวน.....เม็ด

3) ท่านจัดว่าเป็นผู้บริโภคที่ปกติกินอาหาร

☐ เผ็ดเล็กน้อย ☐ เผ็ดปานกลาง ☐ เผ็ดมาก

4) ท่านมีความชอบต่ออาหารเผ็ดเพียงใด

☐ ชอบอย่างยิ่ง

☐ ชอบมาก

☐ ชอบ

☐ ชอบเล็กน้อย

☐ ไม่แน่ใจ

☐ ไม่ชอบเล็กน้อย

☐ ไม่ชอบ

☐ ไม่ชอบมาก

☐ ไม่ชอบมากอย่างยิ่ง

Note: 9 point-hedonic category scale modified from Lawless and Heymann (2010)

Appendix 4.2.2 Interpretation of spicy food consumption questionnaire

According to part (i), consumers were asked to choose on 7-category scales (1 = eat spicy food one time in a year or less, 2 = eat spicy food less than one time per month, 3 = eat spicy food 1-3 times per month, 4 = eat spicy food one time per week, 5 = eat spicy food 3-4 times per week, 6 eat spicy food = every day, and 7 = eat spicy food more than one time per meal per day). For part (ii), the consumers were asked to identify the number of chilli content that they added to a dish. In this case, the most popular Thai dishes, namely Somtam (papaya salad), Yum (Thai dressed salad), Tom yum and Thai noodle (Asia Web Direct, 2012) were chosen as representatives of chilli-containing foods that were consumed in a day. The amounts of chilli content of all dishes were averaged and then were calculated as hotness level of chilli based on the capsaicin content (1.03-1.87 mg/g) of fresh chilli (Prik Keenu; *Capsicum frutescens* L.) which is the most consumed Thai chilli (Botha, 2007). The hotness level was classified base on the range of 0-35,000, 35,000-70,000 and 70,000-160,000 Scoville Heat Units (SHU) as low (score = 1), medium (score = 2) and high heat (score = 3), respectively (Tepsomboon, 1997). In part (iii), consumers were asked to classify themselves in one of the 3 categories according to their chilli eating capacity as light (score = 1), moderate (score = 2) and heavy (score = 3) chilli users. Part (iv), consumers rated on the 9 point hedonic scale according to their like or dislike of chilli taste (1 = dislike extremely to 9 = like extremely). Lastly - the final part (v) for screening participant (as mentioned in Appendix 4.3, Section 4.1), twelve sample sets were presented to the consumers by 3-AFC method. They were asked to choose only one sample that they thought they can notice the hotness differences from three samples of a sample set. The score from 5 items of this questionnaire was combined. The rating score was used to classify the chilli users by the adapted method from Lawless *et al*. (2000).

Appendix 4.3 Questionnaire of panel screening for sensory descriptive analysis (in Thai)

ชื่อ-สกุล:_____ วันที่:_____

เบอร์โทรศัพท์: _____ e-mail: _____

<u>ส่วนที่ 1</u>

กรุณาระบุกลิ่นของตัวอย่างต่อไปนี้ ท่านคิดว่าเป็นกลิ่นใด หากท่านไม่แน่ใจกรุณาอธิบายลักษณะกลิ่นของตัวอย่างตามความรู้สึกของท่านให้มากที่สุดเท่าที่จะเป็นไปได้ โดยเปิดฝาขวดแล้ว ***<u>สูดดม</u>*** ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที

ตัวอย่าง 347 คือกลิ่นของ .....

ตัวอย่าง 519 คือกลิ่นของ .....

ตัวอย่าง 156 คือกลิ่นของ .....

ตัวอย่าง 472 คือกลิ่นของ .....

ตัวอย่าง 798 คือกลิ่นของ .....

ตัวอย่าง 178 คือกลิ่นของ .....

ตัวอย่าง 863 คือกลิ่นของ .....

<u>ส่วนที่ 2</u>

กรุณาชิมตัวอย่างต่อไปนี้ โดยกลั้วตัวอย่างในปากให้ทั่วเป็นเวลา 15 นาที แล้วบ้วนทิ้งในภาชนะที่เตรียมไว้ให้ โดยใช้น้ำสะอาดกลั้วล้างปากอีกทีหนึ่ง

ตัวอย่างชุดที่ 1 ความเข้มมากที่สุด รหัสตัวอย่าง.....
ความเข้มระดับที่ 2 รหัสตัวอย่าง.....
ความเข้มระดับที่ 3 รหัสตัวอย่าง.....
ความเข้มระดับที่ 4 รหัสตัวอย่าง.....

ตัวอย่างชุดที่ 2 ความเข้มมากที่สุด รหัสตัวอย่าง.....
ความเข้มระดับที่ 2 รหัสตัวอย่าง.....
ความเข้มระดับที่ 3 รหัสตัวอย่าง.....
ความเข้มระดับที่ 4 รหัสตัวอย่าง.....

ตัวอย่างชุดที่ 3 ความเข้มมากที่สุด รหัสตัวอย่าง.....
ความเข้มระดับที่ 2 รหัสตัวอย่าง.....
ความเข้มระดับที่ 3 รหัสตัวอย่าง.....
ความเข้มระดับที่ 4 รหัสตัวอย่าง.....

ตัวอย่างชุดที่ 4 ความเข้มมากที่สุด รหัสตัวอย่าง.....
ความเข้มระดับที่ 2 รหัสตัวอย่าง.....
ความเข้มระดับที่ 3 รหัสตัวอย่าง.....
ความเข้มระดับที่ 4 รหัสตัวอย่าง.....

ส่วนที่ 3

3.1 กรุณาอธิบาย "***คุณลักษณะของตัวอย่าง***" ที่รับรู้ได้โดยการชิม การชิมตัวอย่างทำได้โดยกลั้วตัวอย่างในปากให้ทั่ว และบ้วนทิ้งในภาชนะที่เตรียมไว้ให้ หลังจากนั้นใช้น้ำสะอาดกลั้วล้างปากอีกครั้ง แล้วบันทึกคุณลักษณะของตัวอย่างลงในแบบสอบถาม

ตัวอย่างที่ 1 รหัสตัวอย่าง.....

1. ความรู้สึกที่รับรู้ได้ในปาก/รสชาติ

   .....

   .....

2. ถ้ากำหนดให้ "**ความเผ็ด**" มากที่สุดเท่ากับ 10 ตัวอย่าง (รหัสตัวอย่าง) มี "**ความเผ็ด**" เท่ากับเท่าไร (โปรดระบุ).....

ตัวอย่างที่ 2 รหัสตัวอย่าง.....

1. ความรู้สึกที่รับรู้ได้ในปาก/รสชาติ

   .....

   .....

2. ถ้ากำหนดให้ "**ความเผ็ด**" มากที่สุดเท่ากับ 10 ตัวอย่าง (รหัสตัวอย่าง) มี "**ความเผ็ด**" เท่ากับเท่าไร (โปรดระบุ).....

เปรียบเทียบความเหมือน และความแตกต่างระหว่าง ตัวอย่าง (รหัสตัวอย่าง) และ (รหัสตัวอย่าง)

ความเหมือน

.....

.....

ความแตกต่าง

.....

.....

3.2 กรุณาอธิบาย "***คุณลักษณะของตัวอย่าง***" ที่รับรู้ได้โดยการดม การดมตัวอย่างทำได้โดยเปิดฝาขวดแล้ว ***สูดดม*** ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที แล้วบันทึกคุณลักษณะของตัวอย่างลงในแบบสอบถาม

ตัวอย่างที่ 1 รหัสตัวอย่าง.....

3. ความรู้สึกที่รับรู้ได้ในปาก/รสชาติ

   .....

   .....

4. ถ้ากำหนดให้ "**กลิ่นฉุน**" มากที่สุดเท่ากับ 10 ตัวอย่าง (รหัสตัวอย่าง) มี "**กลิ่นฉุน**" เท่ากับเท่าไร (โปรดระบุ).....

ตัวอย่างที่ 2 รหัสตัวอย่าง.....

3. ความรู้สึกที่รับรู้ได้ในปาก/รสชาติ

   .....

   .....

4. ถ้ากำหนดให้ “**กลิ่นฉุน**” มากที่สุดเท่ากับ 10 ตัวอย่าง (รหัสตัวอย่าง) มี “**กลิ่นฉุน**” เท่ากับเท่าไร (โปรดระบุ).....

เปรียบเทียบความเหมือน และความแตกต่างระหว่าง ตัวอย่าง (รหัสตัวอย่าง) และ (รหัสตัวอย่าง)

ความเหมือน

.....

.....

.....

ความแตกต่าง

.....

.....

.....

ส่วนที่ 4

4.1 ทดสอบความแตกต่างลักษณะความเผ็ด โดยวิธี 3-AFC

ชื่อ-สกุล:_____ วันที่:_____

เบอร์โทรศัพท์: _____ e-mail: _____

คำชี้แจง: ให้ทำการทดสอบโดย ***การอม*** เป็นเวลา 15 วินาที จากนั้นคายตัวอย่างทิ้ง รอ 30 วินาที แล้วประเมินตัวอย่าง ท่านจะได้รับตัวอย่างจำนวน 3 ตัวอย่าง สองตัวอย่างมีความเหมือนกันและอีกหนึ่งตัวอย่างมีความแตกต่างให้ทำการทดสอบตัวอย่างตามลำดับที่นำเสนอจากซ้ายไปขวา โดยวงกลมคำตอบที่ท่านคิดว่ามี **“ลักษณะความเผ็ด”** แตกต่างจากอีก 2 ตัวอย่าง หากรู้สึกล้าจากการทดสอบให้พักสักครู่แล้วค่อยทดสอบต่อไป และกรุณาล้างปากด้วยน้ำเปล่าจำนวน 5 ครั้ง ก่อนทดสอบตัวอย่างถัดไป

ชุดทดสอบที่.....	**ตัวอย่างที่ 1**	**ตัวอย่างที่ 2**	**ตัวอย่างที่ 3**
	.....	**.....**	**.....**

4.2 ทดสอบความแตกต่างลักษณะกลิ่นฉุน โดยวิธี 3-AFC

ชื่อ-สกุล:_____ วันที่:_____

เบอร์โทรศัพท์: _____ e-mail: _____

คำชี้แจง: ให้ทำการทดสอบโดย ***การสูดดม*** ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที ท่านจะได้รับตัวอย่างจำนวน 3 ตัวอย่าง สองตัวอย่างมีความเหมือนกันและอีกหนึ่งตัวอย่างมีความแตกต่างให้ทำการทดสอบตัวอย่างตามลำดับที่นำเสนอจากซ้ายไปขวา โดยวงกลมคำตอบที่ท่านคิดว่ามี **"ลักษณะกลิ่นฉุน"** แตกต่างจากอีก 2 ตัวอย่าง หากรู้สึกล้าจากการทดสอบให้พักสักครู่แล้วค่อยทำการทดสอบต่อ ดมทิชชูทุกครั้งก่อนทดสอบตัวอย่างถัดไป

ชุดทดสอบที่.....	**ตัวอย่างที่ 1**	**ตัวอย่างที่ 2**	**ตัวอย่างที่ 3**
	.....	.....	.....

Appendix 4.4 Questionnaire for descriptive analysis (in Thai)

4.4.1 Questionnaire for describing sensory attributes

ชื่อ-สกุล:_____ วันที่:_____

เบอร์โทรศัพท์: _____ e-mail: _____

ส่วนที่ 1

คำชี้แจง: กรุณาชิมตัวอย่าง ด้วยวิธี ***การอม*** เป็นเวลา 15 วินาที จากนั้นคายตัวอย่างทิ้ง รอ 30 วินาที และจาก ***การสูดดม*** ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที แล้วเขียนอธิบายลักษณะทางประสาทสัมผัสของตัวอย่างที่ได้จากการชิมและดม หากรู้สึกล้าจากการทดสอบให้พักสักครู่แล้วค่อยทำการทดสอบต่อ ล้างปากด้วยน้ำหวาน 1 ครั้ง และตามด้วยน้ำเปล่าจำนวน 5 ครั้ง ก่อนทดสอบตัวอย่างถัดไป

รหัสตัวอย่าง	ลักษณะทางประสาทสัมผัสและคำอธิบาย
.....	....
.....	....
.....	....
.....	....
.....	....
.....	....
.....	....

ส่วนที่ 2 กรุณาเปรียบเทียบลักษณะทางประสาทสัมผัสที่เหมือนหรือแตกต่างกันภายในกลุ่มตัวอย่าง

ลักษณะที่เหมือนกัน

....

.....

ลักษณะที่แตกต่างกัน

....

.....

4.4.1 Questionnaire for panel training

4.4.1.1) The 10 cm-Labelled Magnitude Scale (LMS)

ชื่อ-สกุล:_____ วันที่:_____

เบอร์โทรศัพท์: _____ e-mail: _____

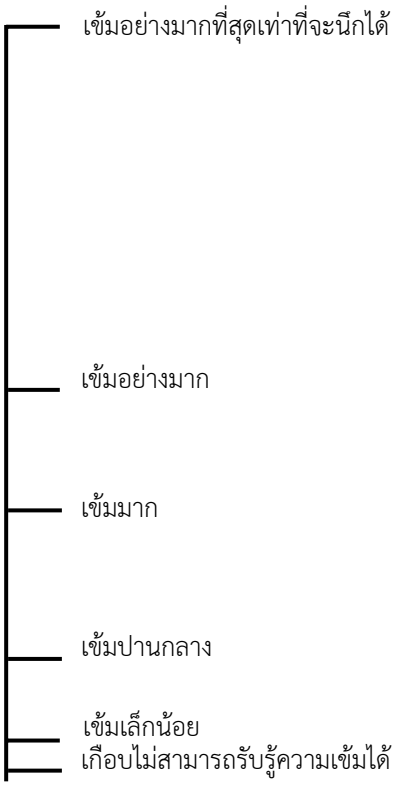
คำชี้แจง (ข้อ 1-2) กรุณาทดสอบตัวอย่างโดย ***<u>การสูดดม</u>*** ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที แล้วบอกระดับความเข้มในแต่ละ***<u>ลักษณะกลิ่น</u>***โดยขีดเส้นลงบนสเกลข้างล่างนี้ เมื่อรู้สึกเหนื่อยล้า ให้หยุดพัก สักครู่ แล้วค่อยประเมินตัวอย่างต่อไป และกรุณาดมกระดาษทิชชูระหว่างตัวอย่าง

1. **กลิ่นฉุนขึ้นจมูก** นิยามคือ กลิ่นฉุนพริกจนระคายเคืองขึ้นจมูกขณะดมตัวอย่าง

2. กลิ่นฉุนแสบจมูก นิยามคือ กลิ่นฉุนพริกจนรู้สึกแสบจมูกขณะดมตัวอย่าง

คำชี้แจง (ข้อ 3-6) กรุณา ***ชิม*** ตัวอย่าง โดย ***<u>การอม</u>*** ตัวอย่างเป็นเวลา 15 วินาที แล้วประเมินความเข้มของแต่ละลักษณะขณะที่ตัวอย่างอยู่ในปาก โดยขีดเส้นลงบนสเกลข้างล่างนี้ จากนั้นคายตัวอย่างทิ้ง เมื่อรู้สึกเหนื่อยล้า ให้หยุดพัก สักครู่ แล้วค่อยประเมินตัวอย่างต่อไป และกรุณาล้างปากด้วยน้ำหวาน 1 ครั้ง และตามด้วยน้ำเปล่าจำนวน 5 ครั้ง ก่อนทดสอบตัวอย่างถัดไป

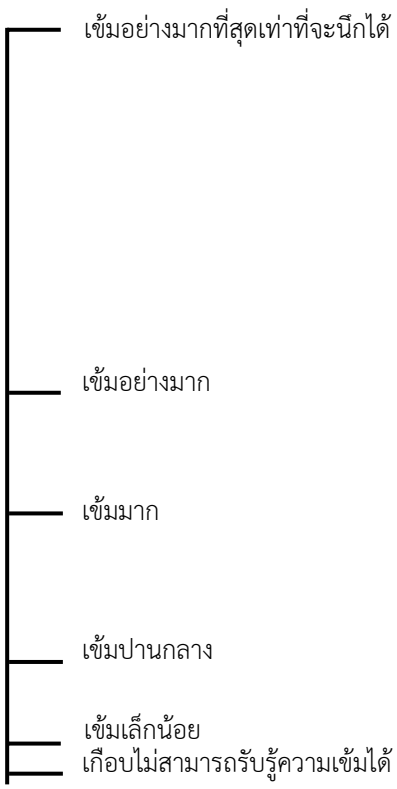
3. ความรู้สึกอุ่น นิยามคือ ความรู้สึกอุ่นขณะที่ตัวอย่าง**อยู่ในปาก**



4. ความเผ็ดร้อน นิยามคือ ความรู้สึกเผ็ดร้อนในปาก เหมือนปากไหม้พองขณะที่ตัวอย่าง**อยู่ในปาก**

5. ความเผ็ดแสบ นิยามคือ ความรู้สึกเจ็บแสบในปาก เหมือนมีเข็มทั่วปาก ซึ่งเกิดจากความเผ็ดขณะที่ตัวอย่าง**อยู่ในปาก**

6. ลิ้นชา นิยามคือ ความรู้สึกชาที่ลิ้นหลังจากคายตัวอย่าง



4.4.1.2) The 10 cm-Unstructured Line Scale (ULS)

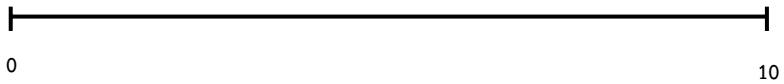
ชื่อ-สกุล:_____ วันที่:_____

เบอร์โทรศัพท์: _____ e-mail: _____

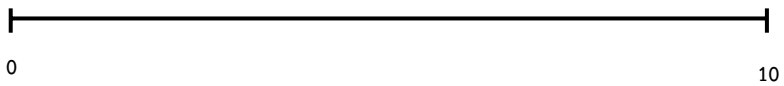
คำชี้แจง (ข้อ 1-2) กรุณาทดสอบตัวอย่างโดย ***การสูดดม*** ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที แล้วบอกระดับความเข้มในแต่ละ ***ลักษณะกลิ่น*** โดยขีดเส้นลงบนสเกลข้างล่างนี้ เมื่อรู้สึกเหนื่อยล้า ให้หยุดพัก สักครู่ แล้วค่อยประเมินตัวอย่างต่อไป และกรุณาดมกระดาษทิชชูระหว่างตัวอย่าง

หมายเหตุ: 0 = ไม่สามารถรับรู้ความเข้มได้ และ 10 = เข้มมากที่สุดเท่าที่จะนึกได้

1. **กลิ่นฉุนขึ้นจมูก** นิยามคือ กลิ่นฉุนพริกจนระคายเคืองขึ้นจมูกขณะดมตัวอย่าง



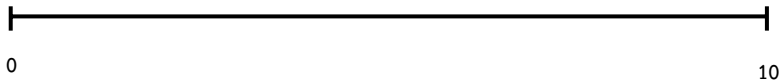
2. **กลิ่นฉุนแสบจมูก** นิยามคือ กลิ่นฉุนพริกจนรู้สึกแสบจมูกขณะดมตัวอย่าง



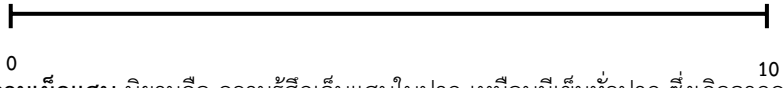
คำชี้แจง (ข้อ 3-6) กรุณา***ชิม***ตัวอย่าง ด้วยการ ***การอม*** ตัวอย่างเป็นเวลา 15 วินาที แล้วประเมินความเข้มของแต่ละลักษณะขณะที่ตัวอย่างอยู่ในปาก โดยขีดเส้นลงบนสเกลข้างล่างนี้ จากนั้นคายตัวอย่างทิ้ง เมื่อรู้สึกเหนื่อยล้าให้หยุดพัก สักครู่ แล้วค่อยประเมินตัวอย่างต่อไป และกรุณาล้างปากด้วยน้ำหวาน 1 ครั้ง และตามด้วยน้ำเปล่าจำนวน 5 ครั้ง ก่อนทดสอบตัวอย่างถัดไป

หมายเหตุ: 0 = ไม่สามารถรับรู้ความเข้มได้ และ 10 = เข้มมากที่สุดเท่าที่จะนึกได้

3. **ความรู้สึกอุ่น** นิยามคือ ความรู้สึกอุ่นขณะที่ตัวอย่าง**อยู่ในปาก**



4. **ความเผ็ดร้อน** นิยามคือ ความรู้สึกเผ็ดร้อนในปาก เหมือนปากไหม้พองขณะที่ตัวอย่าง**อยู่ในปาก**



5. **ความเผ็ดแสบ** นิยามคือ ความรู้สึกเจ็บแสบในปาก เหมือนมีเข็มทั่วปาก ซึ่งเกิดจากความเผ็ดขณะที่ตัวอย่าง**อยู่ในปาก**

6. **ลิ้นชา** นิยามคือ ความรู้สึกชาที่ลิ้นหลังจากคายตัวอย่าง

4.4.2 Questionnaire for sample evaluation (in Thai)

4.4.2.1) Questionnaire for sample evaluation in hotness and pungent attributes (with nose-clip)

ชื่อ-สกุล:_____ วันที่:_____

เบอร์โทรศัพท์: _____ e-mail: _____

คำชี้แจง กรุณาทดสอบตัวอย่างโดย ***การดู*** ตัวอย่าง แล้วบอกระดับความเข้มในแต่ละลักษณะโดยขีดเส้นลงบนสเกลข้างล่างนี้ เมื่อรู้สึกเหนื่อยล้า ให้หยุดพัก สักครู่ แล้วค่อยประเมินตัวอย่างต่อไป

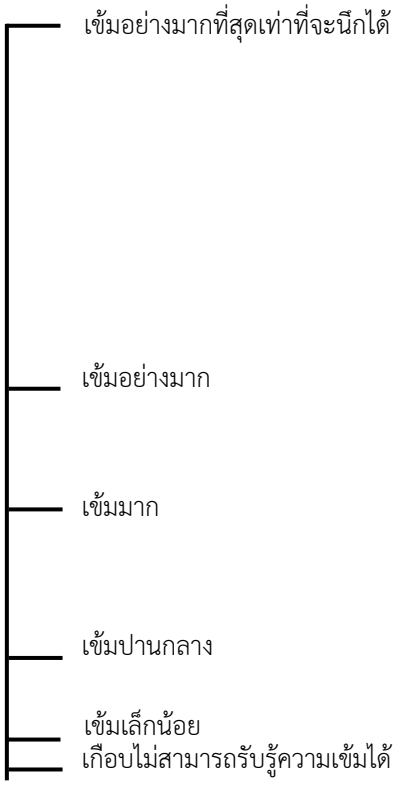
1. **สีแดง** นิยามคือ ลักษณะสีแดงที่มองเห็นด้วยสายตา

คำชี้แจง (ข้อ 2-5) กรุณาทดสอบตัวอย่างโดย ***สูดดม*** ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที แล้วบอกระดับความเข้มในแต่ละ ***ลักษณะกลิ่น*** โดยขีดเส้นลงบนสเกลข้างล่างนี้ เมื่อรู้สึกเหนื่อยล้า ให้หยุดพัก สักครู่ แล้วค่อยประเมินตัวอย่างต่อไป และกรุณาดมกระดาษทิชชูระหว่างตัวอย่าง

2. **กลิ่นพริกสด** นิยามคือ กลิ่นฉุนเหม็นเขียว เหมือนพริกสดและไม่มีกลิ่นหมักเปรี้ยวที่รับรู้ได้ขณะดม
ตัวอย่าง

3. **กลิ่นฉุนขึ้นจมูก** นิยามคือ กลิ่นฉุนพริกจนระคายเคืองขึ้นจมูกขณะดมตัวอย่าง

4. **กลิ่นฉุนแสบจมูก** นิยามคือ กลิ่นฉุนพริกจนรู้สึกแสบจมูกขณะดมตัวอย่าง

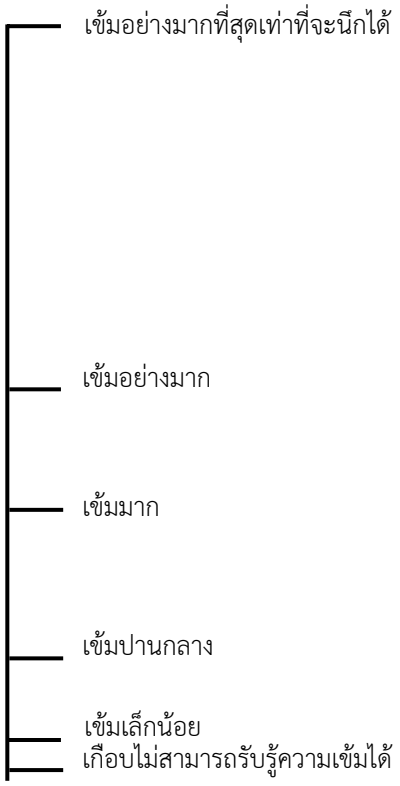


คำชี้แจง (ข้อ 5-9) กรุณา**<u>ปิดจมูกโดยใช้ที่หนีบจมูกที่เตรียมไว้</u>** จากนั้น ***ชิม*** ตัวอย่างโดย ***<u>การอม</u>*** ตัวอย่างเป็นเวลา 15 วินาที แล้วประเมินความเข้มของแต่ละลักษณะขณะที่ตัวอย่างอยู่ในปาก โดยขีดเส้นลงบนสเกลข้างล่างนี้ จากนั้นคายตัวอย่างทิ้ง เมื่อรู้สึกเหนื่อยล้า ให้หยุดพัก สักครู่ แล้วค่อยประเมินตัวอย่างต่อไป และกรุณาล้างปากด้วยน้ำหวาน 1 ครั้ง และตามด้วยน้ำเปล่าจำนวน 5 ครั้ง ก่อนทดสอบตัวอย่างถัดไป

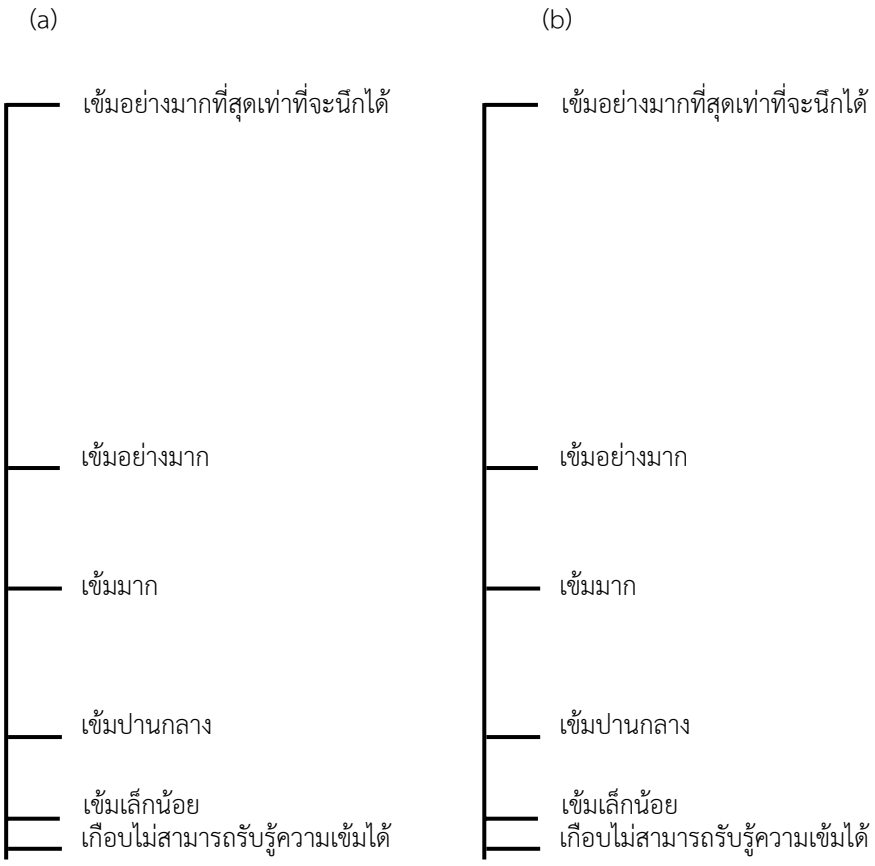
5. ความรู้สึกอุ่น นิยามคือ ความรู้สึกอุ่นขณะที่ตัวอย่าง**อยู่ในปาก** (a) และ**หลังคาย**ตัวอย่าง (b)

(a)

(b)

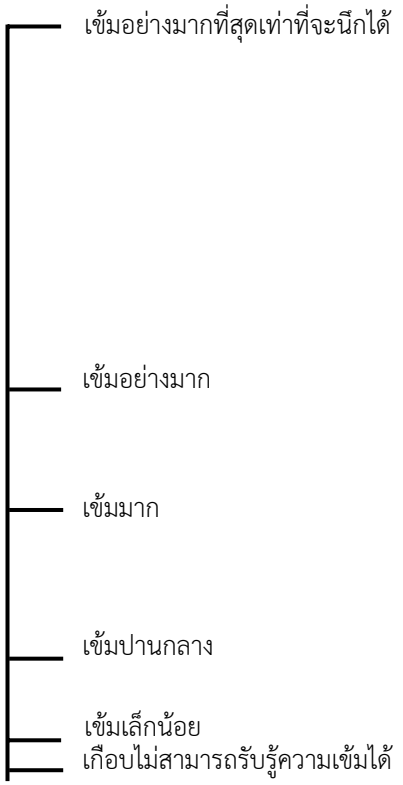


6. **ความเผ็ดร้อน** นิยามคือ ความรู้สึกเผ็ดร้อนในปาก เหมือนปากไหม้พองขณะที่ตัวอย่าง**อยู่ในปาก** (a) และ **หลังคาย**ตัวอย่าง (b)



7. **ความเผ็ดแสบ** นิยามคือ ความรู้สึกเจ็บแสบในปาก เหมือนมีเข็มทั่วปาก ซึ่งเกิดจากความเผ็ดขณะที่ตัวอย่าง**อยู่ในปาก** (a) และ**หลังคาย**ตัวอย่าง (b)

(a)



(b)

8. **ลิ้นชา** นิยามคือ ความรู้สึกชาที่ลิ้นหลังจากคายตัวอย่าง

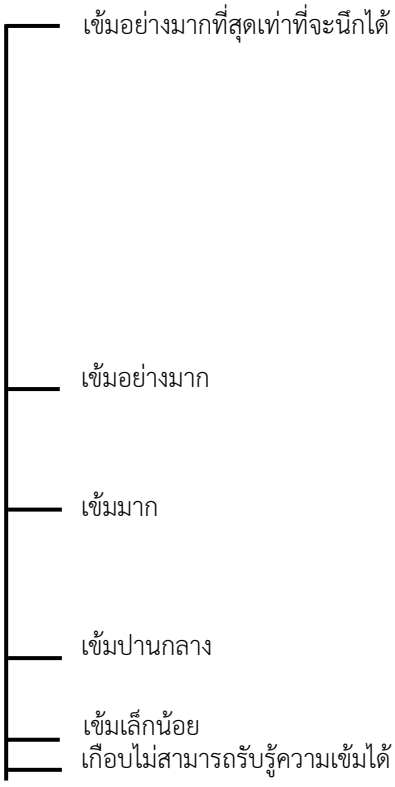
4.4.2.2) Questionnaire for sample evaluation in flavour attributes (without nose-clip)

ชื่อ-สกุล:_____ วันที่:_____

เบอร์โทรศัพท์: _____ e-mail: _____

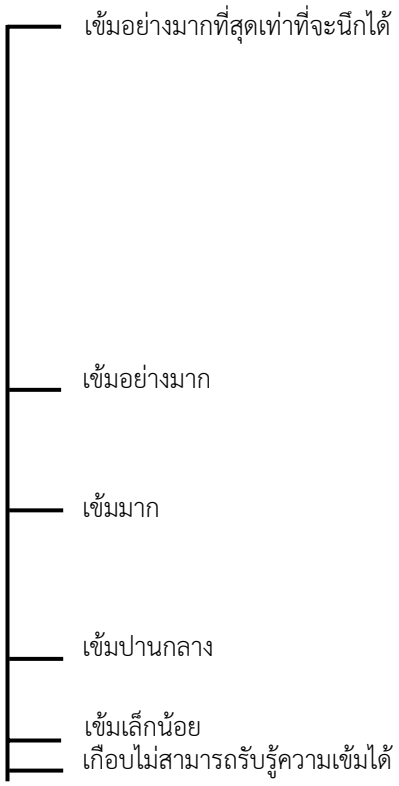
คำชี้แจง กรุณาทดสอบตัวอย่างโดย***การดู***ตัวอย่าง แล้วบอกระดับความเข้มในแต่ละลักษณะโดยขีดเส้นลงบนสเกลข้างล่างนี้ เมื่อรู้สึกเหนื่อยล้า ให้หยุดพัก สักครู่ แล้วค่อยประเมินตัวอย่างต่อไป

1. **สีแดง** นิยามคือ ลักษณะสีแดงที่มองเห็นด้วยสายตา

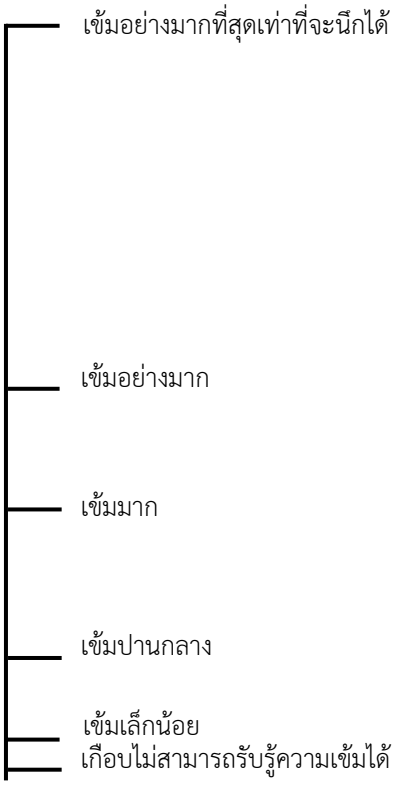


คำชี้แจง (ข้อ 2-9) กรุณา ***ชิม*** ตัวอย่างโดย ***อม ตัวอย่างไว้ในปาก 15 วินาที*** แล้วประเมินความเข้มของแต่ละลักษณะขณะที่ตัวอย่างอยู่ในปาก โดยขีดเส้นลงบนสเกลข้างล่างนี้ จากนั้นคายตัวอย่างทิ้ง เมื่อรู้สึกเหนื่อยล้า ให้หยุดพัก สักครู่ แล้วค่อยประเมินตัวอย่างต่อไป และกรุณาล้างปากด้วยน้ำหวาน 1 ครั้ง และตามด้วยน้ำเปล่าจำนวน 5 ครั้ง ก่อนทดสอบตัวอย่างถัดไป

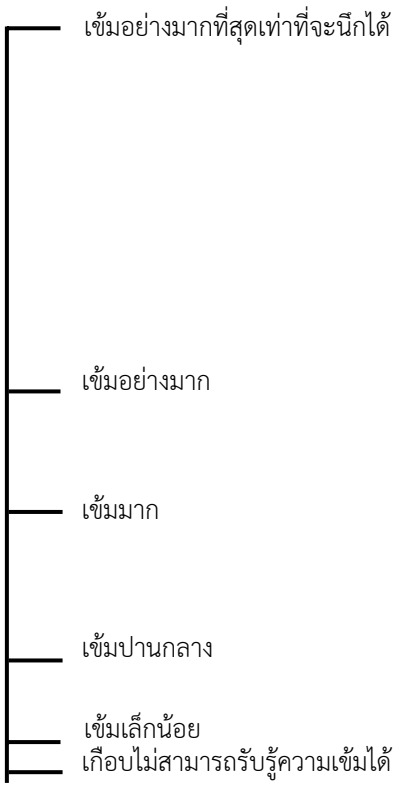
2. กลิ่นพริกสด นิยามคือ กลิ่นฉุนเหม็นเขียว เหมือนพริกสดและไม่มีกลิ่นหมักเปรี้ยวที่รับรู้ได้ขณะดมตัวอย่าง



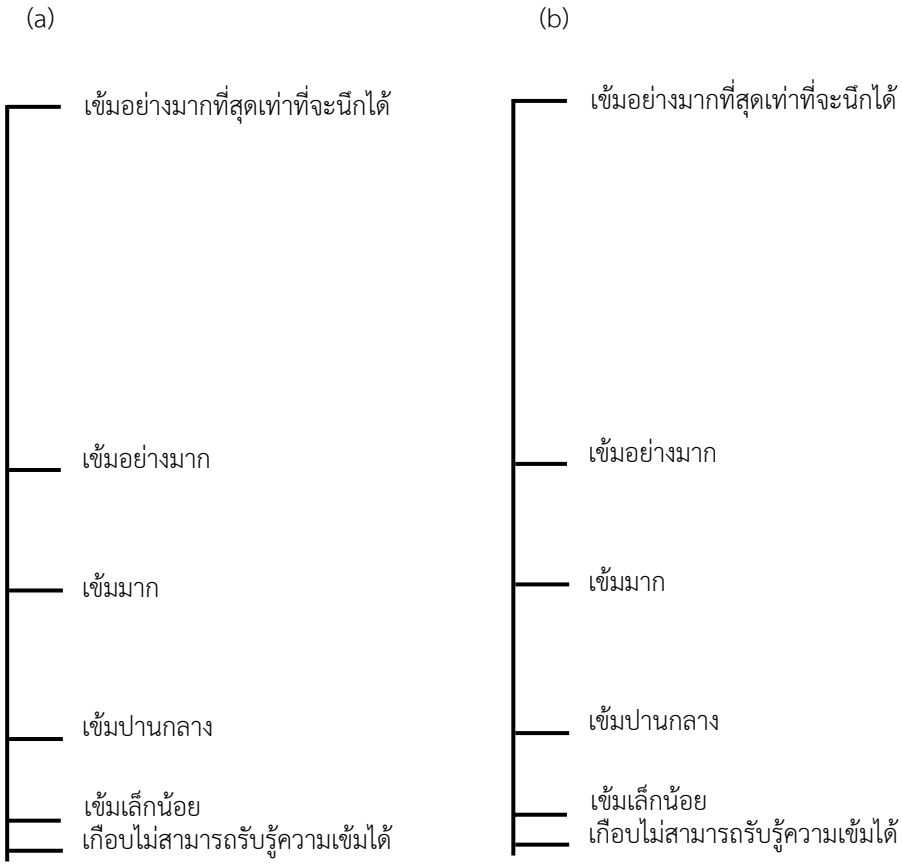
3. กลิ่นฉุนขึ้นจมูก นิยามคือ กลิ่นฉุนพริกจนระคายขึ้นจมูกขณะดมตัวอย่าง



4. กลิ่นฉุนแสบจมูก นิยามคือ กลิ่นฉุนพริกจนรู้สึกแสบจมูกขณะดมตัวอย่าง

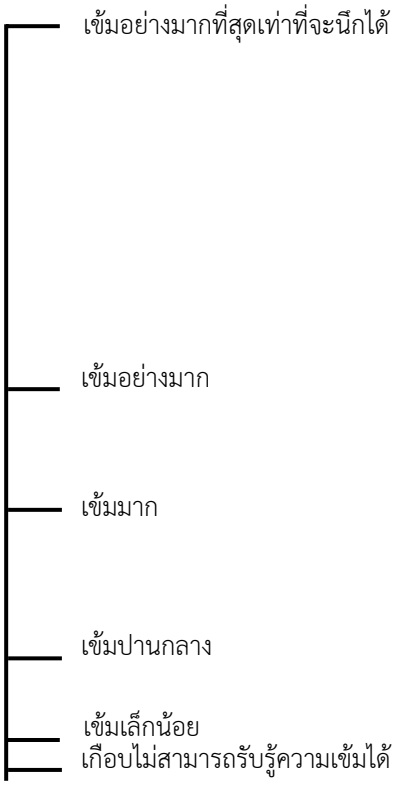


5. ความรู้สึกอุ่น นิยามคือ ความรู้สึกอุ่นขณะที่ตัวอย่าง**อยู่ในปาก** (a) และ**หลังคาย**ตัวอย่าง (b)



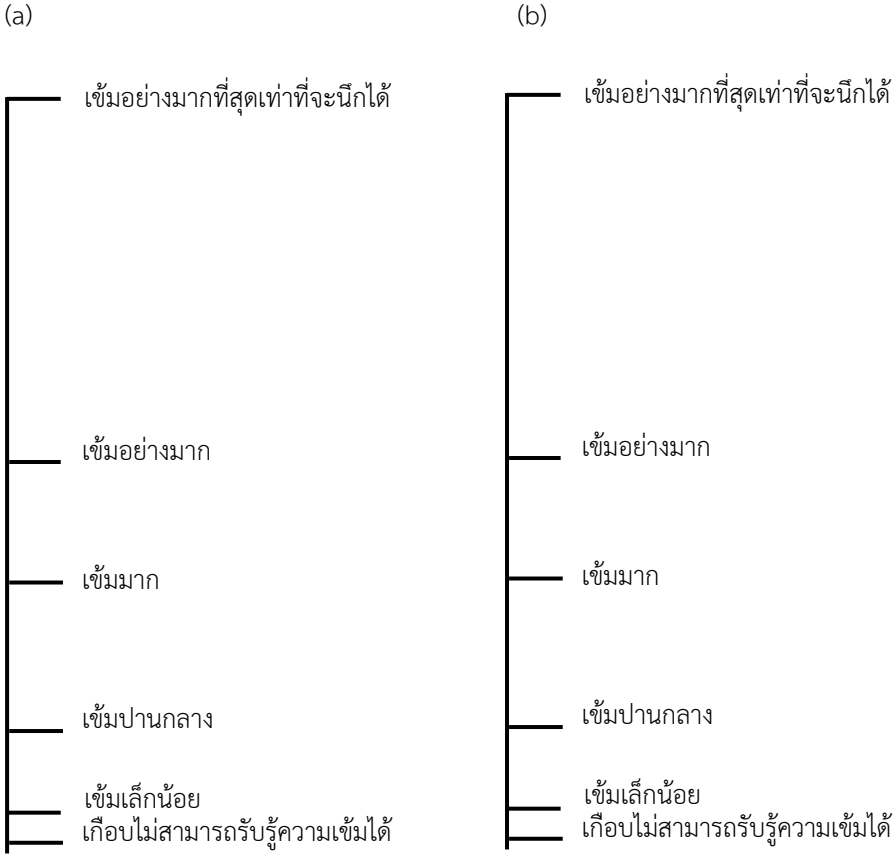
6. ความเผ็ดร้อน นิยามคือ ความรู้สึกเผ็ดร้อนในปาก เหมือนปากไหม้พองขณะที่ตัวอย่าง**อยู่ในปาก** (a) และ**หลังคาย**ตัวอย่าง (b)

(a)



(b)

7. ความเผ็ดแสบ นิยามคือ ความรู้สึกเจ็บแสบในปาก เหมือนมีเข็มทั่วปาก ซึ่งเกิดจากความเผ็ดขณะที่ตัวอย่าง**อยู่ในปาก** (a) และ**หลังคาย**ตัวอย่าง (b)



8. ลิ้นชา นิยามคือ ความรู้สึกชาที่ลิ้นหลังจากคายตัวอย่าง

Appendix 4.5 Questionnaire for threshold measurement (in Thai)

4.5.1 Questionnaire for hotness threshold measurement

ชื่อ-สกุล:_____ วันที่:_____

เบอร์โทรศัพท์: _____ e-mail: _____

ส่วนที่ 1

คำชี้แจง: ให้ทำการทดสอบโดย ***การอม*** เป็นเวลา 15 วินาที จากนั้นคายตัวอย่างทิ้ง รอ 30 วินาที แล้วประเมินตัวอย่าง ท่านจะได้รับตัวอย่างจำนวน 3 ตัวอย่าง สองตัวอย่างมีความเหมือนกันและอีกหนึ่งตัวอย่างมีความแตกต่างให้ทำการทดสอบตัวอย่างตามลำดับที่นำเสนอจากซ้ายไปขวา โดยวงกลมคำตอบที่ท่านคิดว่ามี **"ลักษณะรสชาติ"** แตกต่างจากอีก 2 ตัวอย่าง หากรู้สึกล้าจากการทดสอบให้พักสักครู่แล้วค่อยทำการทดสอบต่อไป และกรุณาล้างปากด้วยน้ำหวาน 1 ครั้ง และตามด้วยน้ำเปล่าจำนวน 5 ครั้ง ก่อนทดสอบตัวอย่างถัดไป

ชุดทดสอบที่.....	**ตัวอย่างที่ 1**	**ตัวอย่างที่ 2**	**ตัวอย่างที่ 3**
	.....	.....	.....

ส่วนที่ 2

ในตัวอย่างชุดนี้มีความแตกต่างของรสชาติอย่างไร.....

4.5.1 Questionnaire for pungent odour threshold measurement

ชื่อ-สกุล:_____ วันที่:_____

เบอร์โทรศัพท์: _____ e-mail: _____

ส่วนที่ 1

A) **คำชี้แจง**: ให้ทำการทดสอบโดย ***การสูดดม*** ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที ท่านจะได้รับตัวอย่างจำนวน 3 ตัวอย่าง สองตัวอย่างมีความเหมือนกันและอีกหนึ่งตัวอย่างมีความแตกต่างให้ทำการทดสอบตัวอย่างตามลำดับที่นำเสนอจากซ้ายไปขวา โดยวงกลมคำตอบที่ท่านคิดว่ามี **"ลักษณะกลิ่น"** แตกต่างจากอีก 2 ตัวอย่าง หากรู้สึกล้าจากการทดสอบให้พักสักครู่แล้วค่อยทำการทดสอบต่อ ดมทิชชูทุกครั้งก่อนทดสอบตัวอย่างถัดไป

ชุดทดสอบที่.....	**ตัวอย่างที่ 1**	**ตัวอย่างที่ 2**	**ตัวอย่างที่ 3**
	.....	.....	.....

ส่วนที่ 2

ในตัวอย่างชุดนี้มีความแตกต่างของกลิ่นอย่างไร.....

Appendix 4.6 Questionnaire for consumer liking tests

4.6.1 Ballot sheet for measurement of overall consumer liking in pungent odour, hotness and flavour of dried chilli samples

วันที่:_____ ID_____

แบบสอบถามการทดสอบวัดความชอบจากผู้บริโภคผลิตภัณฑ์พริกแห้ง

รหัสตัวอย่าง.....

คำชี้แจง ขอให้ท่านผู้ทดสอบประเมินตัวอย่างตามขั้นตอนดังนี้

1. ดมทิชชูสะอาด พักสักครู่ แล้ว สูดดม ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาที แล้วให้ท่านกาเครื่องหมาย √ ในช่องระดับความชอบต่อ***ลักษณะกลิ่นฉุน*** ของตัวอย่าง ตามที่ท่านคิดว่าตรงกับความรู้สึกของท่านมากที่สุด จากนั้นดมทิชชูสะอาดที่เตรียมไว้ให้

ระดับความชอบที่มีต่อ "ลักษณะกลิ่นฉุน"

☐ ชอบอย่างยิ่ง
☐ ชอบมาก
☐ ชอบ
☐ ชอบเล็กน้อย
☐ ไม่แน่ใจ
☐ ไม่ชอบเล็กน้อย
☐ ไม่ชอบ
☐ ไม่ชอบมาก
☐ ไม่ชอบมากอย่างยิ่ง

2. บ้วนบ้วนปากด้วยน้ำสะอาดที่เตรียมไว้ โดยบ้วนใส่โถบ้วน **<u>ปิดจมูกโดยใช้ที่หนีบจมูกที่เตรียมไว้</u>** จากนั้นชิมตัวอย่างผลิตภัณฑ์พริกแห้ง โดย***อมและกลั้วตัวอย่างไว้ในปาก 15 วินาที*** แล้วให้ท่านกาเครื่องหมาย √ ในช่องระดับความชอบต่อ***ลักษณะความเผ็ด*** ของตัวอย่างตามที่ท่านคิดว่าตรงกับความรู้สึกของนั้นมากที่สุด จากนั้นบ้วนตัวอย่างทิ้ง เอาที่ปิดจมูกออก แล้วล้างปากด้วยน้ำหวาน 1 ครั้ง และตามด้วยน้ำสะอาด 5 ครั้ง

ระดับความชอบที่มีต่อ "ลักษณะความเผ็ด"

☐ ชอบอย่างยิ่ง
☐ ชอบมาก
☐ ชอบ
☐ ชอบเล็กน้อย
☐ ไม่แน่ใจ
☐ ไม่ชอบเล็กน้อย
☐ ไม่ชอบ
☐ ไม่ชอบมาก
☐ ไม่ชอบมากอย่างยิ่ง

3. ชิมตัวอย่างโดย ***อมและกลั้วตัวอย่างไว้ในปาก 15 วินาที*** แล้วให้ท่านกาเครื่องหมาย √ ในช่องระดับความชอบต่อ***ลักษณะความเผ็ด*** ของตัวอย่างตามที่ท่านคิดว่าตรงกับความรู้สึกของท่านมากที่สุด จากนั้นบ้วนตัวอย่างทิ้ง แล้วล้างปากด้วยน้ำหวาน 1 ครั้ง และตามด้วยน้ำสะอาด 5 ครั้ง

ระดับความชอบที่มีต่อ “ลักษณะความเผ็ด”

☐ ชอบอย่างยิ่ง
☐ ชอบมาก
☐ ชอบ
☐ ชอบเล็กน้อย
☐ ไม่แน่ใจ
☐ ไม่ชอบเล็กน้อย
☐ ไม่ชอบ
☐ ไม่ชอบมาก
☐ ไม่ชอบมากอย่างยิ่ง

4. เมื่อท่านตอบคำถามครบทั้ง 3 ข้อ แล้ว กรุณาเลื่อนถาดชิมไว้ข้างหน้าบูธทดสอบชิมของท่าน ล้างปากด้วยน้ำสะอาด 5 ครั้ง และดมทิชชูสะอาดที่เตรียมไว้ให้ เพื่อรอทดสอบตัวอย่างถัดไป หากท่านต้องการสิ่งใดเพิ่มเติม กรุณายกมือขึ้นเพื่อส่งสัญญาณให้นักวิจัยทราบ

4.6.2 Ballot sheet for measurement of overall consumer liking in relation to specific threshold intensities of pungent odour and hotness

“ความชอบต่อลักษณะกลิ่นฉุน”

วันที่:_____ ID_____

รหัสตัวอย่าง.....

คำชี้แจง กรุณาดมทิชชูสะอาด พักสักครู่ แล้วสูดดม ตัวอย่าง 1 ครั้ง และกลั้นหายใจเพื่อกักกลิ่นไว้เป็นเวลา 3-5 วินาทีแล้วให้ท่านกาเครื่องหมาย √ ในช่องระดับความชอบต่อ ***ลักษณะกลิ่นฉุน*** ของตัวอย่างแต่ละตัวอย่างตามที่ท่านคิดว่าตรงกับความรู้สึกของท่านมากที่สุด จากนั้นดมทิชชูสะอาดที่เตรียมไว้ให้

ระดับความชอบที่มีต่อ “ลักษณะกลิ่นฉุน”

☐ ชอบอย่างยิ่ง
☐ ชอบมาก
☐ ชอบ
☐ ชอบเล็กน้อย
☐ ไม่แน่ใจ
☐ ไม่ชอบเล็กน้อย
☐ ไม่ชอบ
☐ ไม่ชอบมาก
☐ ไม่ชอบมากอย่างยิ่ง

เมื่อท่านประเมินตัวอย่างเสร็จแล้ว กรุณาเลื่อนถาดชิมไว้ข้างหน้าบูธทดสอบชิมของท่าน ดมทิชชูสะอาดที่เตรียมไว้ให้ เพื่อรอทดสอบตัวอย่างถัดไป หากท่านต้องการสิ่งใดเพิ่มเติม กรุณายกมือขึ้นเพื่อส่งสัญญาณให้นักวิจัยทราบ

“ความชอบต่อลักษณะความเผ็ด”

วันที่:_____ ID_____

รหัสตัวอย่าง.....

คำชี้แจง กรุณาบ้วนปากด้วยน้ำสะอาดที่เตรียมไว้ โดยบ้วนใส่โถบ้วน จากนั้นชิมตัวอย่าง โดย***อมและกลั้วตัวอย่างไว้ในปาก 15 วินาที*** แล้วให้ท่านกาเครื่องหมาย √ ในช่องระดับความชอบต่อ***ลักษณะความเผ็ด*** ของตัวอย่างที่ท่านคิดว่าตรงกับความรู้สึกต่อตัวอย่างนั้นๆ ที่สุด จากนั้นบ้วนตัวอย่างทิ้ง แล้วล้างปากด้วยน้ำหวาน 1 ครั้ง และตามด้วยน้ำสะอาด 5 ครั้ง

ระดับความชอบที่มีต่อ “ลักษณะความเผ็ด”

☐ ชอบอย่างยิ่ง
☐ ชอบมาก
☐ ชอบ
☐ ชอบเล็กน้อย
☐ ไม่แน่ใจ
☐ ไม่ชอบเล็กน้อย
☐ ไม่ชอบ
☐ ไม่ชอบมาก
☐ ไม่ชอบมากอย่างยิ่ง

เมื่อท่านประเมินตัวอย่างเสร็จแล้ว กรุณาเลื่อนถาดชิมไว้ข้างหน้าบูธทดสอบชิมของท่าน ล้างปากด้วยน้ำสะอาด 5 ครั้ง เพื่อรอทดสอบตัวอย่างถัดไป หากท่านต้องการสิ่งใดเพิ่มเติม กรุณายกมือขึ้นเพื่อส่งสัญญาณให้นักวิจัยทราบ

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- The Graduate School, Prince of Songkla University

List of Publication and Proceeding

Publication

Toontom, N., Meenune, M. and Posri, W. 2014. Investigating threshold intensity of hotness and pungent odor perceived by different group of chilli-users. Maejo International Journal of Science and Technology (*Accepted*).

Toontom, N., Meenune, M., Posri W. and Lertsiri, S. 2012. Effect of drying method on physical and chemical quality, hotness and volatile flavour characteristics of dried chilli. International Food Research Journal. 19 (3): 1023-1031.

Conference/Presentation

Toontom, N., Meenune, M. and Posri, W. 2014. Sensory profile analysis of dried Chee fah chilli. Presented at the International Bioscience Conference (IBSC 2014) and the 5th International PSU-UNS Bioscience Conference. September 29-30, 2014. Phuket, Thailand (Poster).

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