Capsaicinoids Profile Identified in Screw Pressed Capsicum Oil Compared with Capsicum Oleoresin

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Abstract

Screw pressed capsicum oil was prepared from *Capsicum annuum* L., jinda and superhot species. This study aimed to determine capsaicin content of screw pressed capsicum oil compared with capsicum oleoresin and their dried powder. Capsaicin was determined using RP-HPLC isocratic mobile phase and the chemical properties of capsicum oil were also determined: acid value, iodine value and peroxide value. HPLC chromatograms of capsicum samples showed that four main capsaicinoids; nor-dihydrocapsaicin, capsaicin, dihydrocapsaicin, and homocapsaicin were identified. Capsaicinoids profile of screw pressed capsicum oil was similar to those of capsaicum oleoresin. Both capsicum oils were of high quality. The highest capsaicin content was found in screw pressed capsicum oil; 2.23 and 2.64 %w/w on dried basis for *Capsicum annuum* L., jinda and superhot, respectively. In addition, capsaicin content of superhot capsicum oil was significantly higher than that of jinda ($p < .05$) in both oil and ethanolic extracts. Screw pressed capsicum oil was statistically significant compared with capsicum oleoresin ($p < .05$). Therefore, screw pressed capsicum oil can be beneficial in agro-food, medical and cosmetic areas.

***Keywords*:** Capsaicin, Capsaicinoids, Screw pressed capsicum oil, Capsicum oleoresin, *Capsicum annuum* L

1. Introduction

Chili (*Capsicum annuum L.*) is a valuable spice which is cultivated in Thailand, Asia, America, Africa and Mediterranean basin. There are several varieties of chili cultivated in Thailand. Horticultural Research Center has bred chili to improve the quality of chili seeds (Suphanburi Agricultural Extension and Development Center) and distributed to farmer for planting. As a result Jinda and Superhot (Chokpachuen et al., 2015) are popular chili species that farmers cultivate for commercial use. Jinda and Superhot are resistant to plant diseases, give relatively big fruit and a spicy taste. In addition, chili breeding was studied to obtain the species with high content of capsaicinoids. There was a report that showed these two species produced relatively high capsaicinoid contents (Thanawiroon, & Homhuan, 2012). Chili has been privatized to different kinds of food in the agro-food industry. In addition, capsicum oleoresin is also used in pharmaceutical manufacturing to relieve pain in topical analgesic.

Capsaicinoids especially capsaicin are well-known active compounds in capsicum. Major capsaicinoids are documented in Thai Herbal Pharmacopoeia (THP) and many articles including nor-dihydrocapsaicin, capsaicin, dihydrocapsaicin and homocapsaicin (Thanawiroon, & Homhuan, 2012). Their chemical structures are showed in Figure 1. Phytochemical studies have reported capsaicin is one of the bioactive compounds in capsicum fruits and seeds. It exhibited anti-oxidant, antimicrobial, antiproliferative, anti-obesity and pain-relieving properties (Smith, & Brooks, 2014). Therefore, the capsicum extract causes interest in pharmaceutical research.

Capsicum oleoresin is the ethanolic extract of dried capsicum fruits which is used as an ingredient in pharmaceutical formulation; for example, ointment, gel, spray (Department of Medical Sciences, 2016b). An attempt to avoid unnecessary solvents from the extraction process, screw pressed oil is an option to get the extract with remaining active components. Screw pressed technique has been used for a long time; for example, coconut oil, rice bran oil, sesame oil. It is more popular due to that this technique can extract most active ingredients in plants and even small insects. Recently the machine has been developed to a smaller one and more user friendly. The machine is one of electric household appliances and is affordable. The other critical step is filtration for the screw pressed technique while solvent extraction may take a longer

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time for extraction, removing solvents and potential risk of residual solvents. Screw pressed capsicum oil is prepared from dried capsicum fruits by a screw pressed machine. Although a lot research has been studied about chili, few articles about capsicum oil have been documented. This study was carried out to determine capsaicinoids in screw pressed capsicum oil compared with capsicum ethanolic extracts and their dried fruit powder.

2. Objectives

The purposes of the study were to determine the capsaicin content between capsicum oil and capsicum oleoresin and to identify some capsaicinoids.

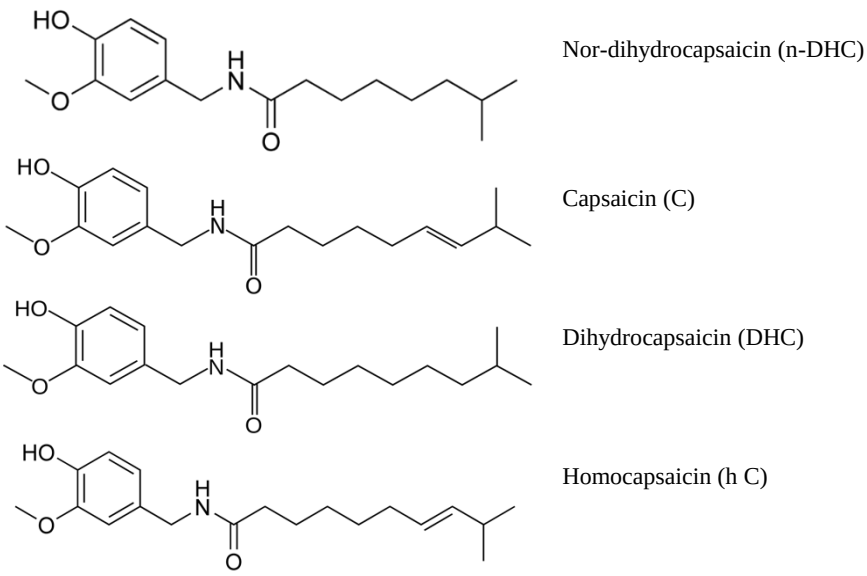

Figure 1 Chemical structures of some capsaicinoids

3. Materials and Methods

3.1 Materials

Dried chili fruits (Superhot) was bought from Chiang Mai province, Thailand and dried chili fruits (Jinda) was bought from Chiang Rai province, Thailand. Only red and ripe chili fruits were selected for this study. These two sources were selected because they cultivated chili for export in Europe and expected low insecticide contamination. They were dried at 60 °C for 2-3 hours to remove some moisture. Standard capsaicin ($C_{18}H_{27}NO_3$) was obtained from Sigma Aldrich, US. 95% of the ethanol is commercial grade. HPLC grade methanol and acetonitrile were bought from Honeywell Burdick & Jackson, Korea. Cyclohexane, isopropanol, benzyl alcohol, formic acid, glacial acetic acid, and Wijs reagent were bought from Carlo Erba, US. Sodium thiosulfate, sodium carbonate, and potassium iodide were bought from Univar. Ultrapure water was prepared by Econoz Milli-Q water purifier system.

3.2 Preparation of screw pressed capsicum oil

Dried chili fruits (27 kg of jinda and 32 kg of superhot) were chopped and prepared with cold press oil using a screw press machine (1 horsepower, Lopburi Province cold press oil group). Capsicum cold press oil was filtered and kept in a glass bottle at room temperature.

3.3 Preparation of capsicum oleoresin

Dried chili powder 30 g, each was extracted with 200 mL of 95% ethanol using a stirrer overnight. The solution was filtered and the residue was the extraction was repeated 2 times. The combined filtration removed ethanol under a vacuum and the temperature of a water bath 50 °C. The ethanolic capsicum extract was kept in a glass bottle until the analysis.

3.4 HPLC Determination of capsaicin

Capsaicin was determined using a reversed phase HPLC system [Agilent 1260, US]. The method was modified according to the chromatographic system of capsicum oleoresin monograph (Department of Medical Sciences, 2017). Column was InfinityLab Poroshell phenyl hexyl C18 (3.0 x 150 mm, 4 μ) and column temperature was 30 °C. Mobile phase composed of 0.1%formic acid in water (pH 2.68-2.74) and acetonitrile (60:40) at flow rate 0.7 mL/minute and run time for 20 minutes. Diode array detector was monitored at 280 nm and injection volume was 10 μL. Software control is EZchrome. Capsaicin content was calculated from its peak area correlated to a standard curve of standard capsaicin. Percent relative peak area of identified capsaicinoids was recorded.

Standard capsaicin was prepared as a stock solution at the concentration of 1,000 μg/mL. Then the standard solution was diluted to 7.5 – 200 μg/mL. Peak areas and their concentrations were plotted at a standard curve and calculated correlation coefficient.

Capsicum oil samples (n=2, each) were prepared in isopropyl alcohol at the concentration of 10 mg/mL. Capsicum oleoresin was prepared at the same procedure except that it was dissolved in methanol. The sample solution was filtered through a syringe filter (0.45 μ) and injected to HPLC in triplicate. Dried capsicum powder was accurately weighed 500 mg (n=2), extracted with 30 mL of methanol, sonicated for 15 minutes and repeated the extraction two times with 10 mL of methanol. After centrifugation, the supernatant was adjusted to 50 mL and filtered through a syringe filter (0.45 μ). The sample solution was injected to HPLC in triplicate.

3.5 Acid value

Capsicum oil was determined acid value by titration method modified from AOAC method No. 940.28 (AOAC, 1990). Accurately weighed 0.5 g of capsicum oil (n=2) and dissolved in a small amount of isopropyl alcohol and filtered to carbon SPE to reduce color and adjusted to 100 mL. The solution was titrated with 0.5 M potassium hydroxide-ethanol VS using phenolphthalein TS as an indicator. The endpoint was light pink. Acid value was calculated as the formula.

$$\text{Acid value (AV)} = \frac{(V_{sample} - V_{blank}) \times M_{KOH\text{-}Ethanol} \times 56.11}{\text{Sample weight (g)}}$$

3.6 Iodine value

Iodine value was determined as Wijs method, AOAC method No. 993.20 (AOAC, 1995a). Capsicum oil was accurately weighed 0.5 g (n=2) in an iodine flask with a stopper. Cyclohexane (20 mL) and Wijs TS (25 mL) were added and swirled. The flask was kept in dark for 30 minutes. Then 20 mL of 10% potassium iodide solution and 100 mL of water were added. Titrated with 0.1 M sodium thiosulfate VS and added 1 mL of starch solution near the endpoint. Iodine value was calculated with the following formula:

$$\text{Iodine value (IV)} = \frac{(V_{blank} - V_{sample}) \times M\ Na_2S_2O_3 \times 12.69}{\text{Sample weight (g)}}$$

3.7 Peroxide value

Peroxide value was determined according to the AOAC method No 965.33 (AOAC, 1995b). Accurately weighed 2 g of capsicum oil (n=2) in an iodine flask and added 30 mL of glacial acetic acid-chloroform (3:2). Then 5 mL of benzyl alcohol and 0.5 mL of freshly prepared saturated potassium iodide solution were added. The flask was swirled for 1 minute and 30 mL of water was added. We titrated the solution with 0.01 M sodium thiosulfate VS. Then 1 mL of starch solution was added near the endpoint.

The dark blue color was clear at the endpoint and resulted in a light yellow color. Peroxide value was calculated with the following formula:

$$\text{Peroxide value (PV), mEq/kg} = \frac{(V_{sample} - V_{blank}) \times M\ Na_2S_2O_3 \times 1000}{\text{Sample weight (g)}}$$

3.8 Statistical analysis

Capsaicin contents between two species of were compared using independent t-test and between screw pressed oil and ethanolic extract using paired t-test with IBM SPSS Statistics version 21.

4. Results

Screw pressed capsicum oil of *C.annuum* L. (Jinda) and *C.annuum* L. (Superhot) was a dark orange-brown color similar to capsicum oleoresin (Figure 2). Acid value, iodine value and peroxide value represented rancidity and the quality of oil (Table 1). Acid value is defined as the weight of potassium hydroxide in mg needed to neutralize free fatty acids present in 1 g of fat or oils. Iodine value is defined as the number of grams of iodine that could be halogenated to 100 g of fat or oils. It measures the unsaturated fatty acids in fat and oils. Peroxide value measures mg equivalent of peroxide oxygen in 1 kg of fat or oils. The high values of the acid value, iodine value and peroxide value represent the potential to cause lipid oxidation and results in rancidity. There is no standard criteria for capsicum oil yet; however, the results showed relatively small values. Therefore, both capsicum oils were of high quality. In comparison, cold-pressing red pepper seed oil showed that iodine value and peroxide value were 5.37±0.07gI_2/100 g oil and 142.52±2.28 mEQ/kg oil, respectively (Chouaibi, Rezig Hamdi, & Ferrari, 2019).

Capsaicin contents of screw pressed showed the highest value compared with their capsicum oleoresin and dried capsicum powder (Table 2). On the other hand, cold-pressing red pepper seed oil showed much lower capsaicin content (3.45±0.05 mg/kg oil = 0.000345 %w/w) (Chouaibi et al., 2019). The capsaicin content was calculated on dried weight of dried capsicum powder. From the loss on drying water contents of dried capsicum powder were 5.81±0.09 and 5.77±0.24 %w/w for Jinda and Superhot, respectively. The capsaicin content of the two kinds of dried capsicum powder was within the THP standard criteria and their contents of ethanolic extracts were not. Capsaicin content of dried chili extracted by solvent has been reported that Jinda dried chili showed 2.38 mg/g dry weight (0.24 %w/w) of capsaicin and 3.24 mg/g dry weight (0.32 %w/w) of dried superhot chili. (Thanawiroon, & Homhuan, 2012). HPLC analytical method of capsaicin was validated according to ICH guideline (ICH, 2005). The results are displayed in Table 3:

(A)  (B) 

Figure 2 (A) Screw pressed capsicum oil (B) Capsicum oleoresin

Table 1 The chemical properties of screw pressed capsicum oil

Test parameters	*C. annuum* L. (Jinda)	*C. annuum* L. (Superhot)
Acid value (mg KOH/g oil)	0.87 ± 0.01	0.16 ± 0.08
Iodine value (g/ 100g oil)	6.05 ± 0.72	7.37 ± 1.54
Peroxide value (mEq/kg oil)	8.73 ± 0.14	3.52 ± 1.20

Table 2 Capsaicin content in dried capsicum powder, capsicum oleoresin and screwed press capsicum oil

Capsicum samples	THP Standard criteria	Capsaicin content (%w/w)		Sig
		C. annuum L. (Jinda)	*C. annuum* L. (Superhot)	
Dried capsicum powder	NLT 0.3% of total capsaicinoids, calculated as capsaicin on the dried basis	0.28 ± 0.01	0.28 ± 0.00	-
Capsicum oleoresin	NLT 6.5% of total capsaicinoids (capsaicin, dihydrocapsaicin, nordihydrocapsaicin), calculated as capsaicin on anhydrous basis	0.70 ± 0.18	1.19 ± 0.27	.005*
Screw pressed capsicum oil	-	2.23 ± 0.01	2.64 ± 0.38	.045*

*p-value < .05 (n=6)

Table 3 HPLC method validation of capsaicin analysis

Parameters						
Linearity (7.5 – 120 µg/mL)	$y = 0.1597x + 0.0053$					
Correlation coefficient	$R^2 = 0.9998$					
Accuracy	%Recovery					
30 µg/mL	98.13 ± 1.33					
60 µg/mL	100.28 ± 0.71					
90 µg/mL	99.74 ± 0.42					
Intraday precision (%RSD)	Day 1	RT	PA	Day 2	RT	PA
30 µg/mL		0.07	1.48		0.17	1.24
60 µg/mL		0.06	0.93		0.08	1.54
90 µg/mL		0.05	1.08		0.07	2.41
Interday precision (%RSD)		RT	PA			
30 µg/mL		0.16	1.77			
60 µg/mL		0.16	1.39			
90 µg/mL		0.23	1.83			

Capsaicinoid profiles of screw pressed capsicum oil and capsicum oleoresin were showed in HPLC chromatograms (Figures 3-5). Nordihydrocapsaicin was eluted first, then capsaicin, dihydrocapsaicin and homocapsaicin, respectively. The relative retention times of these peaks corresponded to 0.90, 1.0 and 1.30 of nordihydrocapsaicin, capsaicin, and dihydrocapsaicin, respectively as indicated in monograph (Department of Medical Sciences, 2017). The relative peak areas of each peak were showed in Table 4 and capsaicin was the highest capsaicinoids found in all samples.

Table 4 Capsaicinoids profile of screw pressed capsicum oil and capsicum oleoresin

Capsaicinoids	Screw pressed capsicum oil*		Capsicum oleoresin*	
	C. annuum L. (Jinda)	*C. annuum* L. (Superhot)	*C. annuum* L. (Jinda)	*C. annuum* L. (Superhot)
Nordihydrocapsaicin (n-DHC)	6.07	5.74	6.92	6.55
Capsaicin (C)	57.22	61.63	60.87	59.72
Dihydrocapsaicin (DHC)	26.28	28.50	27.48	29.81
Homocapsaicin (h C)	10.42	4.14	4.74	3.92

*Percent relative peak area

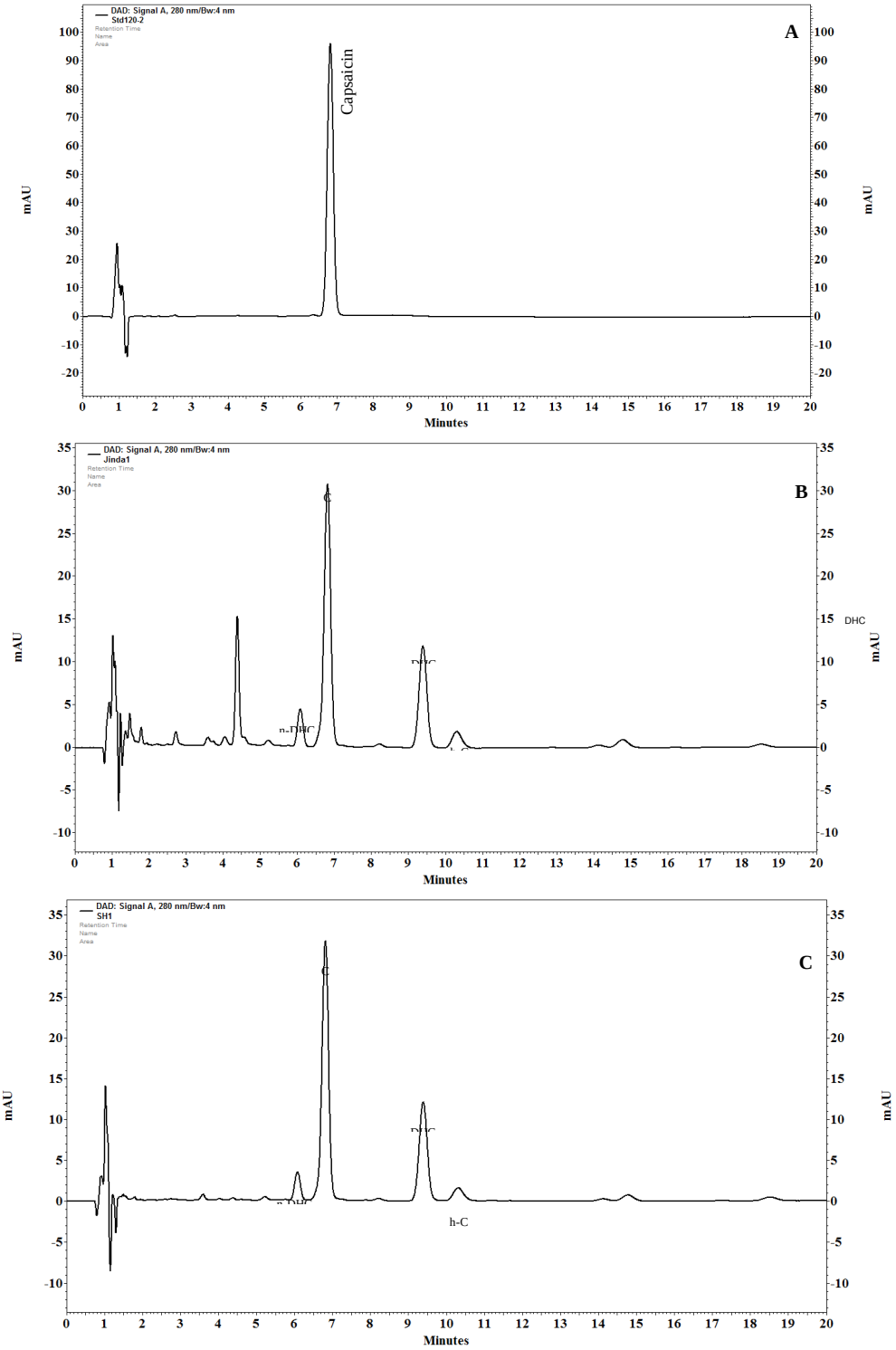

Figure 3 HPLC chromatogram of (A) standard capsaicin at 120 µg/mL and capsicum oil of (B) *C.annuum* L. (Jinda) (C) *C.annuum* L. (Superhot) at the concentration of 10 mg/mL

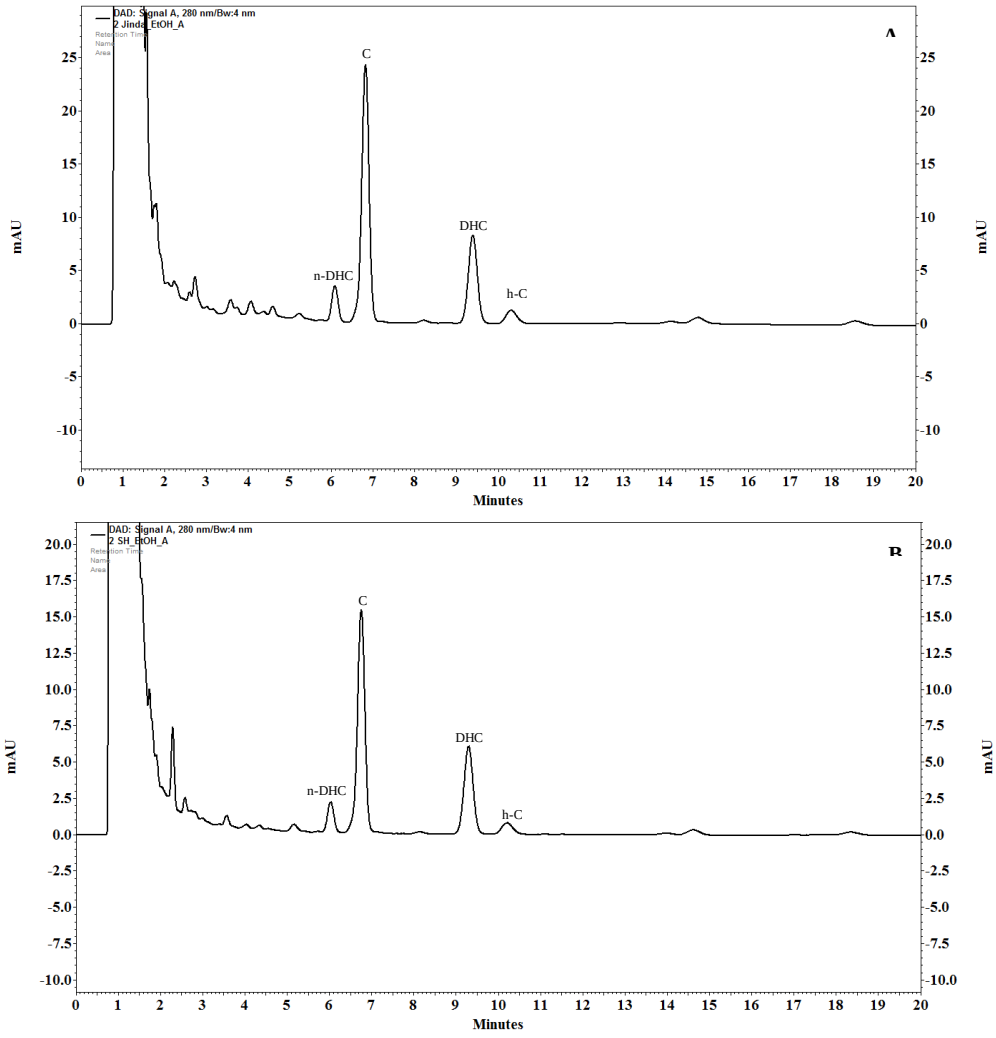

Figure 4 HPLC chromatogram of capsicum oleoresin of (A) *C.annuum* L. (Jinda) (B) *C.annuum* L. (Superhot) at the concentration of 10 mg/mL

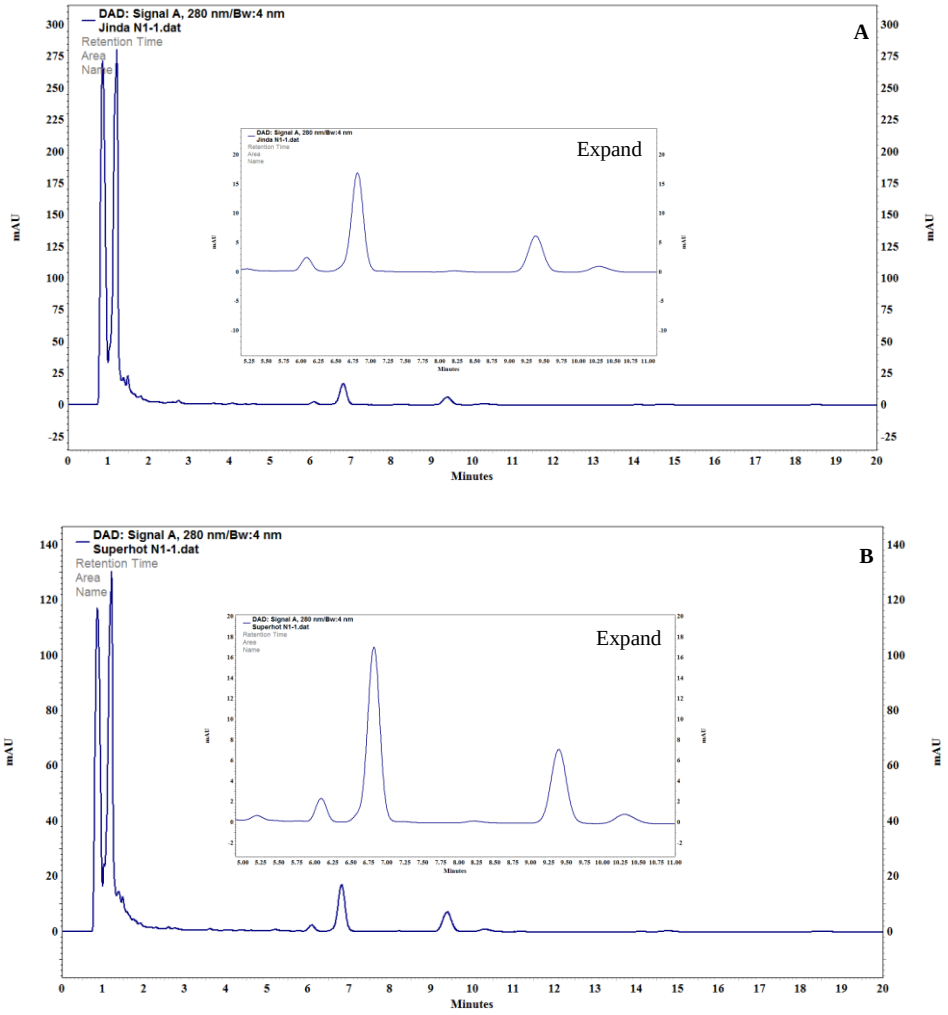

Figure 5 HPLC chromatogram of (A) *C.annuum* L. (Jinda) (B) *C.annuum* L. dried powder at the concentration of 10 mg/mL

5. Discussion

Statistical analysis showed that capsaicin contents of *C. annuum* L. (Superhot) was significantly higher than that of *C. annuum* L. (Jinda) for both screwed pressed oil and ethanolic extracts (Table 2). Screw pressed oil also showed significantly higher capsaicin content than that of capsicum oleoresin from the same sources of capsicum fruits (Table 5). The fixed cost of screw pressed oil and solvent extraction was instrument cost: screw pressed machine and rotary evaporator, respectively. Variable costs were included in the cost of dried chili fruits, labor cost, solvent cost and shipping cost. Starting from dried capsicum fruit gave approximately 2.6-2.7% yield of screw press capsicum oil. In comparison, the ethanolic extract of dried capsicum powder (30 g) yielded about 28.7 – 31.6 %w/w. Although the cost of preparing screw pressed oil was comparable to ethanolic extraction, the same amount of oil gives much higher capsaicin contents than oleoresin. In addition, capsicum oil showed much clearer major capsaicinoids in HPLC chromatograms (Figure 3). However, capsicum oleoresin showed some residual solvent even after solvent evaporation (Figure 4).

Table 5 Paired t-test analysis of capsaicin content between capsicum oil and capsicum oleoresin

Capsaicin	n	Mean ± SD	t	*p*-value
Screw pressed capsicum oil	12	2.43 ± 0.34	-34.130	.000*
Capsicum oleoresin	12	0.95 ± 0.34		

*p-value < .05

6. Conclusion

In Conclusion, determination of capsaicin contents in dried chili powder showed the average content in each species, but the content in capsicum oil and oleoresin showed how much each method can extract most chemical components. Four main capsaicinoids have been identified in screw pressed capsicum oil and capsicum oleoresin; nordihydrocapsaicin, capsaicin, dihydrocapsaicin and homocapsaicin. Capsaicin was the highest capsaicinoids found in both chili species and both screw pressed oil and oleoresin. Capsaicin content of screw pressed capsicum oil showed significantly higher than that of capsicum oleoresin made from the same dried capsicum fruits. Although percent yield of screw pressed capsicum oil was relatively low, it can be improved by selected capsicum specie and the screw pressed technique.

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8. References

AOAC. (1990). Official methods of analysis. 15th ed. AOAC Official Method 940.28 Acid value. Washington D.C: Association of Official Analytical Chemists.

AOAC. (1995)a. Official Methods of Analysis of AOAC International 16th ed.vol.II, Ch.41, AOAC Official Method 993.20 Iodine value of oils and fats, 8-9. Gaitherburg, MD: Association of Official Analytical Chemists.

AOAC. (1995)b. Official methods of analysis of AOAC International 16th ed.vol.II, Ch.41, AOAC Official Method 965.33 Peroxide value of oils and fats, 9. Gaitherburg, MD: Association of Official Analytical Chemists.

Chokpachuen, J., Nimkingrat, T., Tongtip, S., Prasertsuk, A., Tharanugool, P.& Kurubunjerdjit. (2015). Study on Chinda chilli field trial. *The Best Research Department of Agriculture 2015*. Retrieved from http://www.doa.go.th/research/attachment.php?aid=1571

Chouaibi, M., Rezig L., Hamdi, S., Ferrari, G. (2019). Chemical characteristics and compositions of red pepper seed oils extracted by different methods. *Industrial Crops & Products*. *128*, 363-370.

Department of Medical Sciences. (2016)a. *Thai Herbal Pharmacopoeia*. Phrik Khi Nu Monograph. (pp.329-337). The Agricultural Co-operative Federation of Thailand., Ltd: Department of Medical Sciences, Ministry of Public Health.

Department of Medical Sciences. (2016)b. *Thai Herbal Pharmacopoeia*. Capsicum gel Monograph. (pp.338-339). The Agricultural Co-operative Federation of Thailand., Ltd: Department of Medical Sciences, Ministry of Public Health.

Department of Medical Sciences. (2017). *Thai Herbal Pharmacopoeia*. Capsicum oleoresin Monograph. (pp.380-381). The Agricultural Co-operative Federation of Thailand., Ltd: Department of Medical Sciences, Ministry of Public Health.

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). (2005) Validation of Analytical Procedures: Text and Methodology Q2(R1).

Smith, H., & Brooks, J. R. (2014). Capsaicin-based therapies for pain control. In *Capsaicin as a Therapeutic Molecule* (pp. 129-146). Springer, Basel.

Suphanburi Agricultural Extension and Development Center (Plant Tissue Culture). Hybrid chilli "Superhot" *Department of Agriculture Extension.* Retrieved from http://www.aopdt01.doae.go.th/KM/พริกขี้หนูลูกผสม%20ˮ ซุปเปอร์ฮอท˝.pdf

Thanawiroon, C., & Homhuan, S. (2012). Comparison of capsaicinoid content, scoville heat units and total sensory value on different stages of physiological maturity in chilli. *Journal of Science and Technology, Ubon Ratchathani University*. *13*(2), 6-13.