Forced Degradation Study of the Ethanolic Capsicum Extract

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Abstract

Capsicum oleoresin is the ethanolic extract of *Capsicum annuum* L., which contains mainly capsaicinoids (capsaicin, dihydrocapsaicin, nor-dihydrocapsaicin), volatile oil, and fixed oil. It is the ingredient for some capsicum formulations. The objective of this research was to study the degradation conditions of capsicum oleoresin. The content of capsaicin was evaluated using a reversed-phase HPLC. The capsaicinoid profile was also determined. The results showed that the oxidation condition decreased the capsaicin content dramatically as compared to the control sample. Therefore, the process after extraction and storage condition including packaging should be considered to prolong the major components in capsicum oleoresin.

***Keywords*: *Capsicum annuum* L, Capsicum oleoresin, Degradation study, Ethanolic extract**

1. Introduction

Chili or *Capsicum annuum* L. was the raw material to prepare ethanolic capsicum extract or capsicum oleoresin. There are many species of chili in Thailand; for example, superhot, jinda, and others. Superhot was reported to contain a high content of capsaicin (Thanawiroon & Homhuan, 2011). Capsaicinoids are the main components in capsicum oleoresin, and most capsaicinoids found in chili are capsaicin, dihydrocapsaicin, nor-dihydrocapsaicin, and homocapsaicin (Ministry of Public Health. 2017). Capsaicin is the bioactive compound, and it exhibits a pungent and burning sensation and induces skin vasodilation (Sharma, Vij, & Sharma, 2013). Capsaicin acts as an analgesic for neuropathic pain and muscle pains. Also, capsicum oleoresin was used in varieties of topical preparations, namely, gel, cream, and spray. This study focused on the degradation conditions that affected the content of capsaicin.

Examining degradation products under stress conditions is useful for developing and validating suitable analytical procedures. Stress testing evaluated the susceptibility of the substance to hydrolysis, temperature, oxidation, and photolysis (ICH, 2003). Stress testing depends on the individual substance, and capsaicin is the compound of interest in this study. Other capsaicinoids were also determined. It had been reported that high-performance liquid chromatography was a sensitive and reliable analytical method for the quantitative determination of capsaicin (Stipcovich, Barbero, Ferreiro-González, Palma, & Barroso 2017).

Stability study is mandatory for drug products according to ASEAN guidelines (Association of Southeast Asian Nations, 2013). In this study, the ethanolic capsicum extract was designed to study forced degradation conditions to evaluate what is likely to affect the quality, safety and efficacy of the extract. Besides, the more stressful condition can provide particular stability information.

2. Objectives

The objectives of the study were to determine capsaicin contents and capsaicinoid profiles of the ethanolic capsicum extract under forced degradation conditions.

3. Materials and Methods

Materials

Capsicum annuum L.(Superhot) was purchased from Nan province, Thailand. Acetonitrile and methanol were HPLC grade and obtained from Burdick & Jackson, Korea. Ethanol was a commercial grade. Formic acid and hydrogen peroxide were analytical grade and obtained from Fischer Scientific, UK. Hydrochloric acid and sodium hydroxide were purchased from Carlo Erba. Standard capsaicin was obtained from Cato. Ultrapure water was prepared from Puris, a water purifier system.

Methods

3.1 Preparation of ethanolic capsicum extract

Superhot chili was dried and ground to powder. Capsicum powder (20 g) was stirred in 135-mL ethanol overnight. The solvent was filtered and replaced two times more. The ethanol was removed from the combined filtrate under vacuum. Capsicum oleoresin was obtained and determined its capsaicin content and capsaicinoid profile using reversed-phase high-performance liquid chromatography (RP HPLC).

3.2 Degradation conditions

Acid condition

Ethanolic capsicum extract was accurately weighed 100 mg (n=2). 0.1 M Hydrochloric solution (10 mL) was added and kept at room temperature for 15 minutes. The solution was filtered through a syringe filter and injected into HPLC.

Basic condition

Ethanolic capsicum extract was accurately weighed 100 mg (n=2). 0.1 M Sodium hydroxide solution (10 mL) was added and kept at room temperature for 30 minutes. The solution was filtered through a syringe filter and injected into HPLC.

Water hydrolysis

Ethanolic capsicum extract was accurately weighed 100 mg (n=2). Water (2 mL) was added and kept at 100 °C for 5 hours. Then the solution was adjusted to 10 mL with methanol. The solution was filtered through a syringe filter and injected into HPLC.

Heat

Ethanolic capsicum extract was accurately weighed 100 mg (n=2) and kept in the oven at 100 °C for 5 hours. Then the sample was added 10 mL of methanol. The solution was filtered through a syringe filter and injected into HPLC.

Oxidation

Ethanolic capsicum extract was accurately weighed 100 mg (n=2). 30%v/v Hydrogen peroxide solution (10 mL) was added and kept at room temperature for 2 hours. The solution was filtered through a syringe filter and injected into HPLC.

Ultraviolet light

Ethanolic capsicum extract was accurately weighed 100 mg (n=2) and kept under ultraviolet light for 7 days in the stability chamber. Then the sample was added 10 mL of methanol. The solution was filtered through a syringe filter and injected to HPLC

3.3 Determination of capsaicin using RP HPLC

The major components of capsicum oleoresin were analyzed with HPLC [Agilent 1260] and acquired data with OpenLab EZChrom software. The column was Poroshell C18 (3 x 150 mm, 4 μ) and was controlled at 25 °C. Mobile phase composed of acetonitrile and 0.1% formic acid in water in a gradient system, 10% to 100% acetonitrile over 24 minutes, washed the column at 100% acetonitrile for 1 minute and regenerated to 10 % acetonitrile in 4 minutes. The flow rate was 0.5 mL/minute and the total run time was 30 minutes. The chromatogram was monitored at a wavelength of 280 nm. The injection volume was 10 μL

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(n=2). The content of capsaicin was calculated relative to the standard curve of capsaicin (3.12 – 100 µg/mL). The peak areas of other capsaicinoids were also recorded and calculated as percent relative peak areas.

3.4 Method validation

The analytical method was validated for its accuracy, precision, linearity, the limit of detection and limit of quantitation. Standard capsaicin was prepared at the concentration of 1,000 µg/mL and diluted to 7.5, 15, 30, 60, 120 and 200 µg/mL for determination of linearity. The standard curve, equation and correlative coefficient were calculated. The standard capsaicin solution was diluted to 30, 60 and 90 µg/mL and added into the sample for determination of accuracy and precision. Percent recovery was calculated from (the amount found/the amount added)x100. The limit of detection and the limit of quantitation were determined as 3: 1 and 10: 1 signal-to-noise ratio, respectively.

4. Results and Discussion

This analytical method was validated and showed good linearity, accuracy and precision within the criteria of Guideline, 2005 (Table 1). The correlation coefficient of the standard curve of capsaicin was above 0.999. The percent recovery of capsaicin was in the range of 100.55 – 108.50 for standard capsaicin 30-90 µg/mL. This method showed precise and reproducibility. Limit of detection and limit of quantitation were 0.38 and 3.97 µg/mL, respectively.

Table 1 Method validation of capsaicin in capsicum oleoresin

Parameters	**Results**					
Linearity (7.5 – 200 µg/mL)	y = 16851x + 22520					
Correlation coefficient	R^2 = 0.9998					
Accuracy	%Recovery					
30 µg/mL	102.76 ± 4.97					
60 µg/mL	108.50 ± 3.88					
90 µg/mL	100.55 ± 0.97					
Intraday precision (%RSD)	Day 1	RT	PA	Day 2	RT	PA
30 µg/mL		0.00	0.02		0.02	0.02
60 µg/mL		0.00	1.01		0.02	0.05
90 µg/mL		0.03	1.00		0.02	1.31
Interday precision (%RSD)		RT	PA			
30 µg/mL		0.06	0.01			
60 µg/mL		0.04	1.28			
90 µg/mL		0.03	1.14			
LOD (µg/mL)	0.38 ± 0.02					
LOQ (µg/mL)	3.97 ± 0.03					

RT = retention time
PA = peak area

The percent yield of ethanolic capsicum extract was 41.39 by weight of dry powder. The average capsaicin content was 0.99±0.00 %w/w. After the sample was treated in forced degradation conditions, their capsaicin contents were determined and showed in Table 2. ANOVA showed that the capsaicin contents were decreased statistically in these stress conditions except for the condition under UV light. There was a study of thermal degradation of capsaicin in red chili which showed that the higher temperature and the longer drying time, the lower the capsaicin content kinetically (Arifin & Djaeni, 2018). Besides, the freeze-drying method can preserve capsaicin content more than other drying methods (sundry, hot air dry and microwave vacuum dry) for chili (Maurya, Gothandam, Ranjan, Shakya, & Pareek, 2018). Similarly, the ethanolic capsicum extract, which was heat in the oven showed a decrease in capsaicin content compared with the

control extract in this study. In opposite, the stability study of pure capsaicin showed no degradation under basic hydrolysis, UV light, wet and dry heat (Shrivastava & Saxene, 2011).

Table 2 Capsaicin content of ethanolic capsicum extract at various degradation conditions

Conditions	%Capsaicin (%w/w)	Sig
Control	0.99 ± 0.00	-
Acid hydrolysis	0.63 ± 0.04	.039*
Basic hydrolysis	0.53 ± 0.16	.010*
Water hydrolysis	0.60 ± 0.01	.024*
Heat	0.60 ± 0.00	.024*
Oxidation	0.18 ± 0.05	.000*
UV light	0.68 ± 0.08	.078

**The mean difference is significant at the 0.05 level.*

Main capsaicinoid compounds were eluted in a similar pattern, as shown in HPLC chromatogram (Figures 2 and 3). Nor-dihydrocapsaicin eluted first and capsaicin, followed by dihydrocapsaicin and homocapsaicin. The sample that was oxidized with 30% hydrogen peroxide showed a dramatic decrease in capsaicin content but increasing in dihydrocapsaicin content as shown in the capsaicinoid profile (Figure 4).

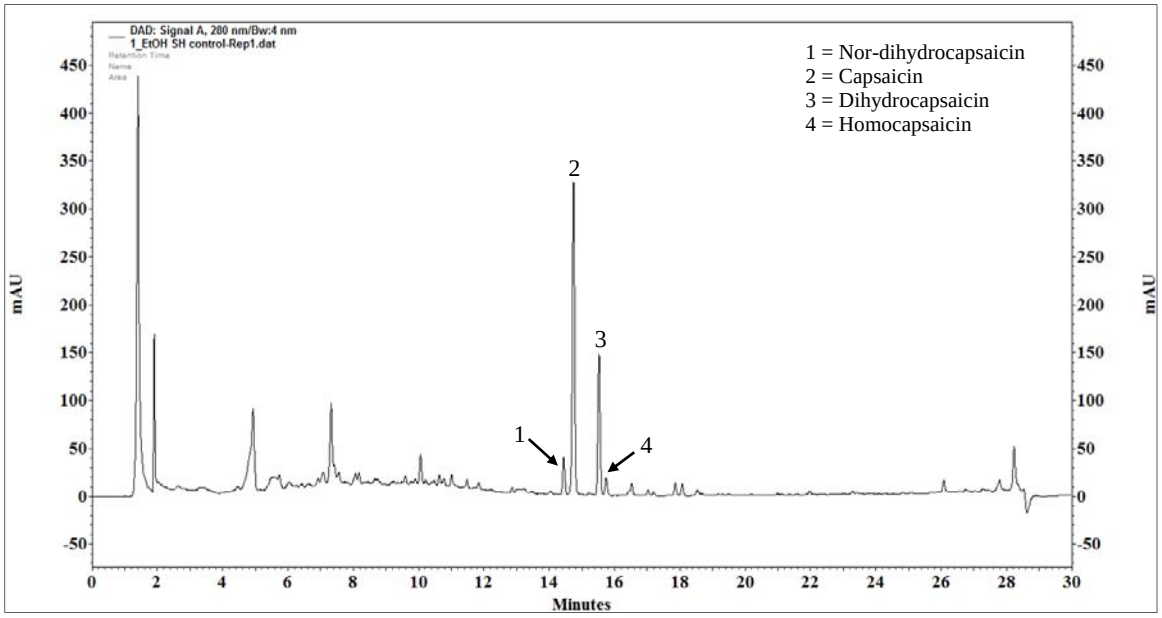

Figure 1 HPLC chromatogram of ethanolic capsicum extract

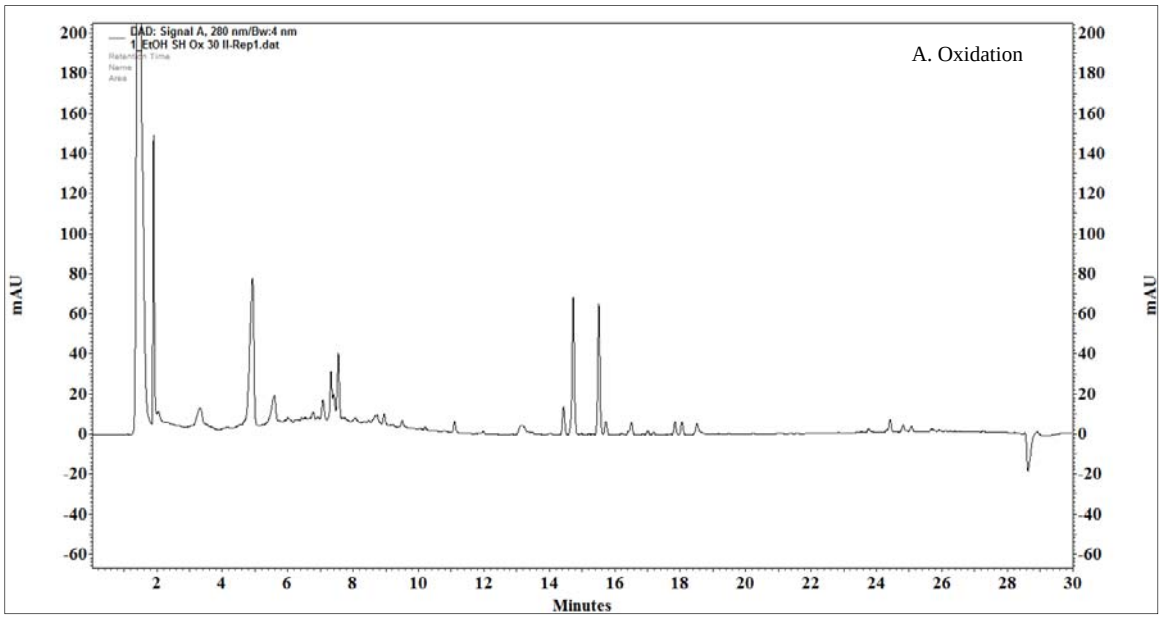

Figure 2 HPLC chromatogram of ethanolic capsicum extract after oxidation

From HPLC chromatogram, the analytical method can separate capsaicin from other capsaicinoids and other degraded products with accepted resolution. The capsaicin contents were degraded 31.31-81.82% in the forced degradation conditions. Capsaicin was degraded mostly in oxidation and basic hydrolysis while it was relatively stable under UV light. It could be the ethanol residue in capsicum oleoresin that may expedite the degradation in such harsh conditions in this study.

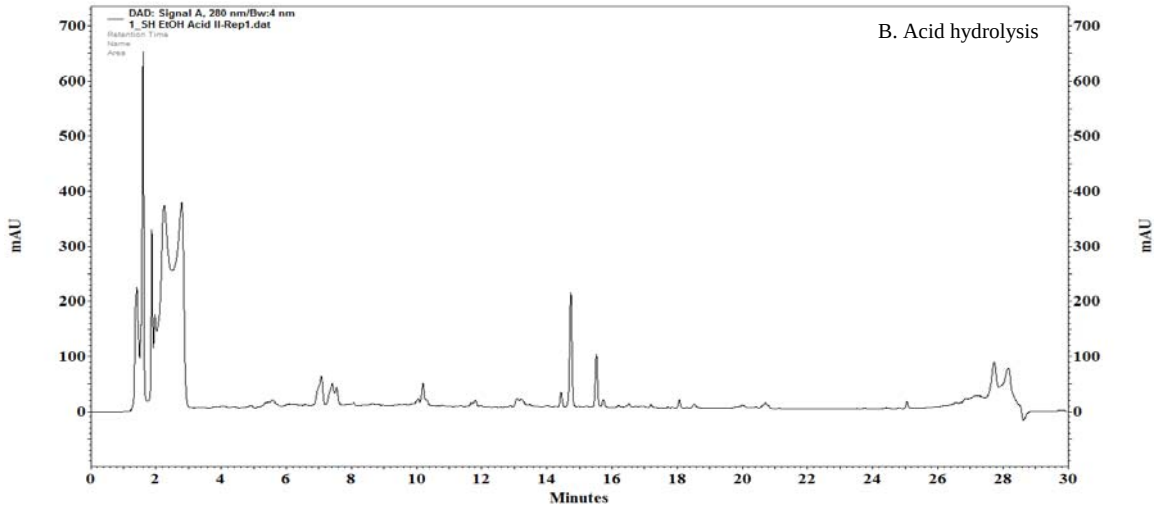

Figure 3 HPLC chromatogram of ethanolic capsicum extract after forced degradation at various conditions

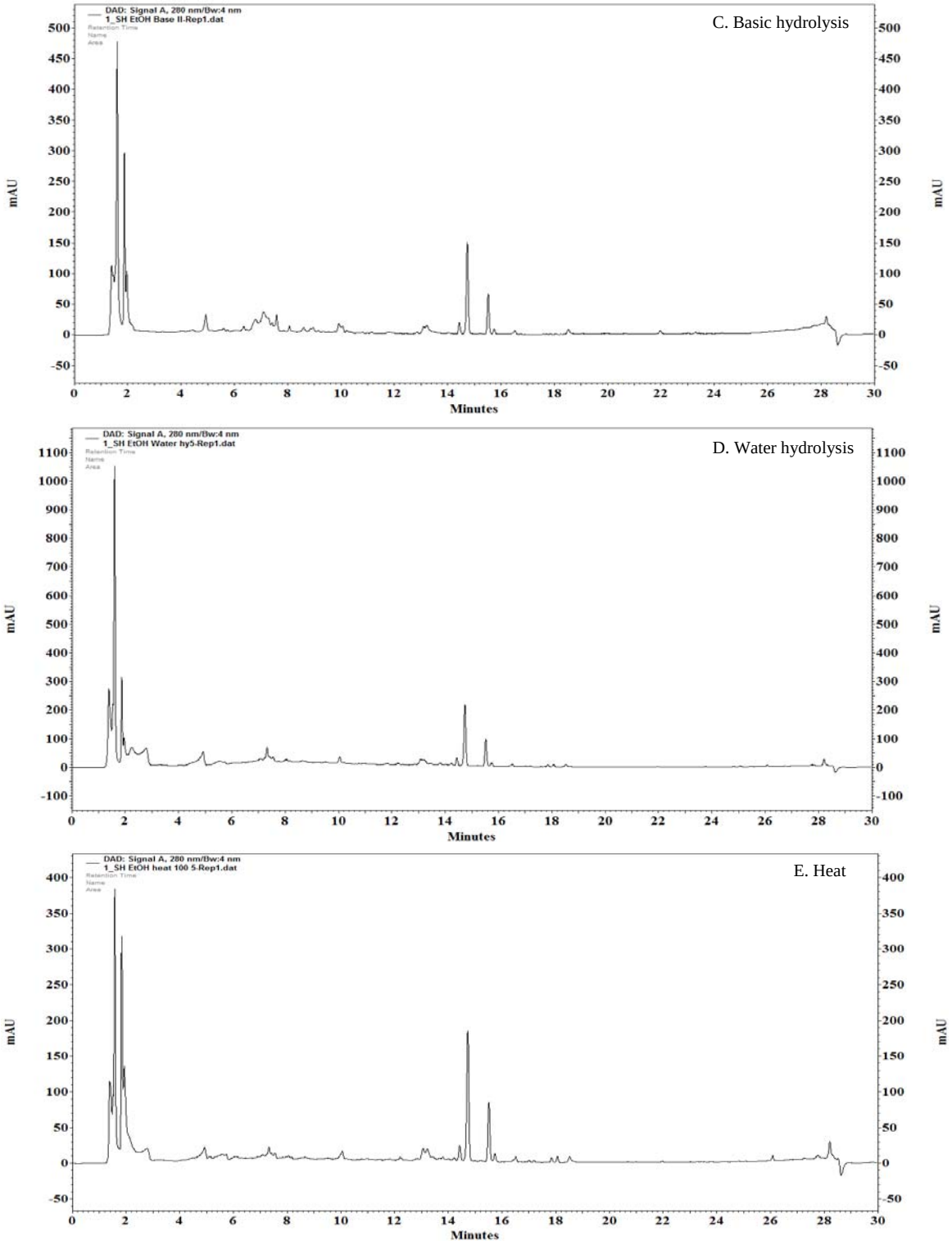

Figure 3 (continued) HPLC chromatogram of ethanolic capsicum extract after forced degradation at various conditions

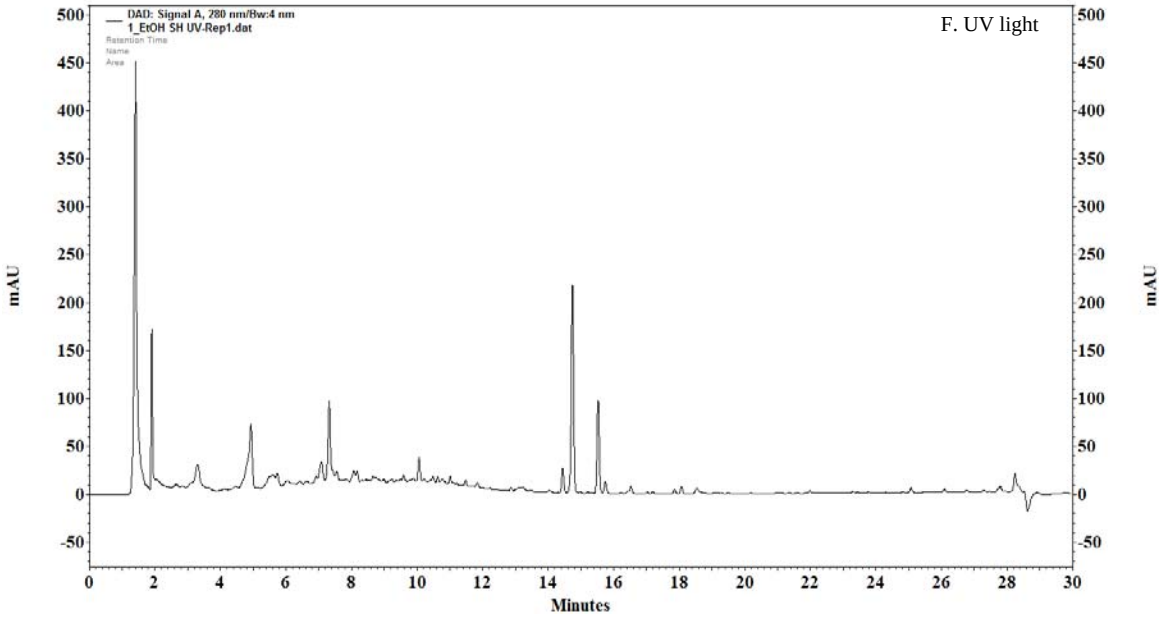

Figure 3 (continued) HPLC chromatogram of ethanolic capsicum extract after forced degradation at various conditions

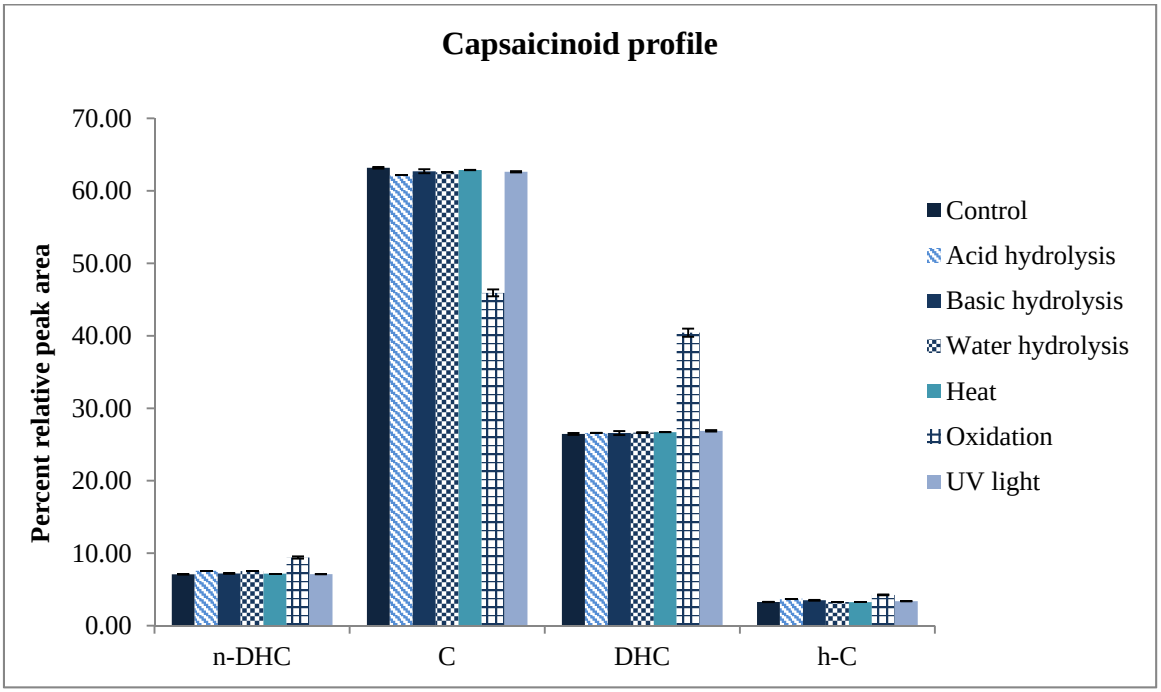

Figure 4 Graphic picture of capsaicinoids profile

5. Conclusion

This study showed that the HPLC analytical method was validated and can discriminate the main capsaicinoids from the degradation products. Capsicum oleoresin was degraded most in oxidation and basic hydrolysis while it showed less degraded under acid and water hydrolysis, heat, and UV light. Capsaicinoid profile showed a low amount of capsaicin whereas dihydrocapsaicin was increased and slightly increased in nor-dihydrocapsaicin and homocapsaicin under oxidation condition, respectively. Therefore, capsicum oleoresin should be dried as much as possible after solvent extraction and kept at room temperature and in light protecting container.

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