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Original Article

# Rapid determination of capsaicinoids by colorimetric method

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ARTICLE INFO

*Article history:*
Received 5 July 2016
Received in revised form
3 November 2016
Accepted 4 November 2016
Available online 8 December 2016

*Keywords:*
capsaicinoids
colorimetric method
liquid–liquid extraction
solid–liquid extraction

ABSTRACT

Capsaicinoids, the pungent component of chili peppers, are generally analyzed by precise analytical techniques, such as gas chromatography and high-performance liquid chromatography (HPLC), but these are not practical for the mass analyses of samples. To analyze mass samples rapidly, a colorimetric method was suggested. In this work, pigments and capsaicinoids were efficiently separated from chili pepper extract by sequential solid–liquid extraction and liquid–liquid extraction in test tubes followed by a colorimetric analysis on the capsaicinoids by a selective chromogenic reaction with Gibbs reagent (2,6-dichloroquinone-4-chloroimide). In the comparison of the capsaicinoid content by the colorimetric method and HPLC using acetone extracts of fresh pepper and dry red pepper as samples, $R^2$ was 0.9973 and 0.9816, respectively, which shows a high linear correlation. In addition, a minimum of 1 μg/mL capsaicinoids can be detected and it was therefore determined that the method can efficiently analyze a great quantity of samples in a short time.



## 1. Introduction

The secondary metabolites retaining the pungency of chili peppers, called capsaicinoids, are alkaloids composed of vanillylamide and an acyl chain, which are classified by the acyl chain structure into a capsaicin group, dihydrocapsaicin group, and *N*-vanillyl-*n*-acrylamide group [1]. The biocomponents of capsaicinoid derivatives from chili peppers are affected by various factors such as varieties of chili pepper, cultivation conditions, level of aging, and processing methods [2–5]. Among capsaicinoid derivatives, capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-*N*-vanillylnonanamide) account for 80–90% of the capsaicinoids in chili peppers and are therefore the main determinants of pungency [6–10].

The annual global output of chili peppers was approximately 34.5 million tons in 2012 [11] and this is cultivated as a spice crop. Chili peppers are used in natural pigments and drug substances, and have a high economic value. Thus, rapid and simple quantification of the capsaicinoid content is very

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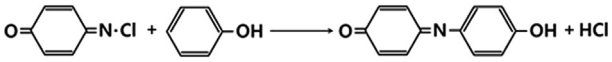
http://dx.doi.org/10.1016/j.jfda.2016.11.007

important for breeding a variety of chili peppers and quality control of processed products.

Various methods to measure the capsaicinoid content have been used. The pungency in chili pepper fruit or food is traditionally measured by the Scoville heat test [12], which gradually dilutes a sample to measure pungency. It quantifies the heat level and is a useful scale to indicate pungency. However, this method is dependent on human senses and requires trained personnel. It can be difficult to measure the pungency of food, such as kimchi groups, that utilizes a lot of spices. Instrumental analytical methods to quantify pungency include high-performance liquid chromatography (HPLC) [13–16], gas chromatography [17–19], and UV spectrophotometry [20]. The aforementioned chromatographic analysis can measure the amount of capsaicinoid derivatives and total capsaicinoids. However, it requires a lot of time, money, effort, and equipment so it is inefficient to apply to the breeding of chili peppers or to the quality control of processed products where a large quantity of samples needs to be analyzed quickly. Meanwhile, the Scoville heat unit (SHU) uses a universal unit indicating pungency, and therefore the amount of capsaicinoids analyzed by instrumental analysis is sometimes converted to SHU [21].

2,6-Dichloroquinone-4-chloroimide (DCQ) forms color by reaction with phenols and can be used to detect capsaicinoids. However, pigments contained in chili pepper include capsanthin, carotenoids, chlorophyll, etc., [22,23] and pepper extracts




cannot be directly quantified by the colorimetric method. To overcome these disadvantages, colorimetry has been suggested for the evaluation of extracted capsaicinoids and pigments after separating them from a chili pepper extract by thin-layer chromatography (TLC) or paper chromatography [24,25].

Capsaicinoids share a phenolic hydroxyl group in the molecular structure. A phenolic hydroxyl group forms phenolate ions in basic conditions, and forms salt with metal ions. In this process, changes in basicity of a solution affect relative solubilities in a solvent and so capsaicinoids can be selectively transferred from an organic solvent to an aqueous solution or from an aqueous solution to an organic solvent.

Liquid–liquid extraction is used to separate compounds based on the difference in relative solubilities between two solvents, such as a water-immiscible solvent and a water-miscible solvent, which are not mixed together. In this study, pigments and capsaicinoids were selectively separated from chili pepper extract in test tubes based on the principles of solid–liquid extraction and liquid–liquid extraction. The vanillyl group of capsaicinoids was reacted with DCQ for color formation to suggest a colorimetric method able to quantitatively measure total capsaicinoid content.

## 2. Materials and Methods

### 2.1. Materials

Capsaicin, dihydrocapsaicin, ethanol, DCQ, HPLC-grade acetonitrile, and HPLC-grade water were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Chili peppers (*Capsicum annuum*) were cultivated on a farm near Chuncheon, Korea. We obtained fresh pepper from the farm and dry red pepper was purchased on sale.

### 2.2. Extraction of capsaicinoids

Five-gram whole fresh peppers and dry red pepper were ground with a home blender for 3 minutes and then a fivefold volume of acetone was added, respectively, to the extract at 50°C for 1 hour in triplicate. Centrifuged supernatant was taken for HPLC and colorimetric analysis.

### 2.3. HPLC and TLC analysis

For HPLC analysis, the acetone extract was filtered with a 0.22-μm membrane filter and then directly injected into the HPLC system (GTS 30, Young Lin, Anyang-si, Republic of Korea) using YMC hydrosphere $C_{18}$ S-5 (4.6 × 150 nm) as a column. The isocratic mobile phase was acetonitrile/1% acetic acid in water (40:60, v/v) with a flow rate of 1.0 mL/min. The absorbance was measured at 280 nm (see Supplementary Material online). For TLC, silica gel 60 $F_{254}$ precoated plates (Merck, Darmstadt, Germany) were used with toluene/chloroform /acetone (55/26/19, v/v/v) in the solvent system. Capsaicinoids were detected by spraying 0.1% DCQ solution and placing the plate in a chamber saturated with ammonia vapor.

### 2.4. Experimental design

For direct colorimetric quantification of capsaicinoids from pepper extracts, these pigments act as interfering substances so it is important to separate the pigments and capsaicinoids. When pepper extracts are developed by TLC, there are several components affecting chromogenic reactions, besides pigments. To investigate the partition efficiency of capsaicinoids from an organic solvent layer to an alkali solution, 250 μg capsaicin was dissolved in 5 mL of dichloromethane (polarity 3.1), tetrachloromethane (polarity 1.6), and *n*-hexane (polarity 0.0), which are hydrophobic organic solvents with different polarities. Then, 10 mL of 0.05N NaOH was added to each capsaicin solution followed by intense vortexing. Finally, capsaicin transferred into a water layer was identified by TLC.

Because of liquid–liquid extraction between an *n*-hexane solution and NaOH solution, capsaicin is solubilized to be an NaOH solution for complete partitioning. Therefore, the *n*-hexane solution obtained through solid–liquid extraction was used for liquid–liquid extraction with an NaOH solution. A 4-mL *n*-hexane solution containing capsaicinoids and pigments was taken and then partitioned by 0.05N NaOH solution (10 mL).

### 2.5. Colorimetric quantification of capsaicinoids

Chromogenic substances by origin are water-soluble components with high polarity extracted with acetone and they affect the chromogenic reactions of capsaicinoids. To remove them, extracts in the test tube were completely dried and then *n*-hexane was added to selectively extract capsaicinoids and pigments from the remaining solids; 1 mL acetone extract of