Original Research Article

Simple Spectrophotometric Method for determination of Capsaicin in chilis and spicy sauces.

ABSTRACT

A simple extractive-spectrophotometric method for the determination of Capsaicin in chilis and spicy sauces was developed. The spectral behavior of Capsaicinoids (CAP) in the micellar system of surfactants was investigated. An analytical method is proposed for the determination of CAP based on the use of Adogen-464 in toluene and measurement of the absorbance of the organic extract at 286 nm. The calibration graph was linear from 4 to 44 ⌈g mL-1 of CAP; the detection and quantification limits were 0.259 and 0.865 ⌈g ml-1, respectively. The method was applied to analysis of Capsaicinoids in several commercial and natural food products.

Keywords:
Analytical methods, capsaicinoids, micellar system, organic extracts, spectral behavior.

1. INTRODUCTION

Paprika (Capsicum annuum) is a fruit used as a spice to add taste to foods or as a colorant for foods or cosmetics and as raw material to the pharmaceutical industry worldwide. The pungency of paprika is caused by capsaicin, one of the named capsaicinoids (vanillyl amides of fatty acids) (Fig. 1), which are present in paprika varieties in different amounts (Vinha and Haas, 1997). Capsaicinoids are responsible for the pungent flavor in many "hot" foods (Cooper et al., 1991). Therefore, generally, one distinguishes through pungency, i.e., capsaicin content (Vinha and Haas, 1997). Since the effect they produce is an irritation to the nerve endings responsible for heat and pain sensation in the mouth, standardization of the level of heat is essential (Cooper et al., 1991).

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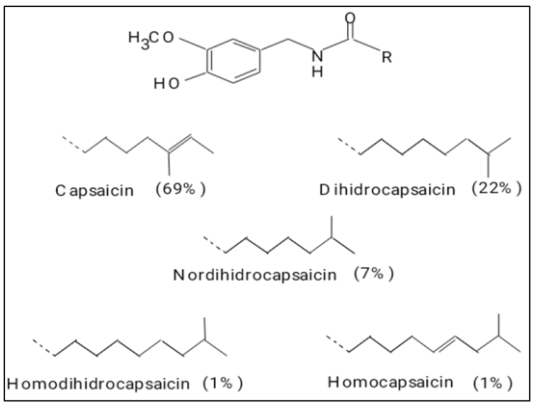

Figure 1. Capsaicinoids structures and percentage of abundance

For most of the products containing capsaicinoids, it is crucial to know the content of each of those. Most of the current methods proposed for estimating total capsaicinoids are impractical. They are very time-consuming, require expensive and sophisticated instrumentation, and provide impractical precision, considering natural products' variability (Rymal et al., 1984).

A rapid method of determining capsaicin content of capsicum fruits is desirable so that plant breeders, growers, seed companies, and especially pepper processors must be able to quickly estimate the relative pungency of their raw products to have reasonable quality assurance in their finished products (Bajaj, 1980). The first reported reliable measurement of chile pungency is the Scoville Organoleptic Test. This test uses a taste panel of five individuals who evaluate a chile sample and then record the hot flavor level. The sample is then diluted until pungency can no longer be detected. This dilution is referred to as the Scoville Heat Unit (Collins et al., 1995). Historically, the food industry has preferred the organoleptic method since it is a direct measure of levels of heat (Cooper et al., 1991). The disadvantage of this test is that it is subjective, and members of the taste panel cannot determine the amount of each of the capsaicinoids present in the sample (Collins et al., 1995). In this organoleptic procedure, the trained buyers going into a grower's field, tasting the raw fruit, and relying on their subjective skill and taste memory to characterize the pepper crop's pungency; alternatively, systematic taste panels may use the threshold-dilution or Scoville method. A problem with organoleptic procedures is that capsacinoids result in the desensitization of taste receptors in the mouth and panelists become "burned out" rapidly (Rymal et al., 1984).

Several colorimetric and spectrophotometric methods for the determination of capsaicin are available. Most of the methods are less than ideal because of color instability and turbidity formation. In other spectrophotometric approaches, elaborate procedures make them inconvenient for routine analysis. Processes involving diazonium salts as reagents require the preparation of these in situ because of their instability. In contrast, methods that involve the measurement of spot size or visual comparison of color are only semiquantitative (Bajaj, 1980).

Spectrophotometric methods involve reactions of capsaicinoids with either vanadium oxytrichloride or phosphomolybdic acid to produce colored species. Although not specific for capsaicinoids, the assay will give a result that is proportional to the amount of heat (Cooper et al., 1991). Also, a variety of GC and HPLC methods have been proposed (Cooper et al., 1991; Krajewska and Powers, 1987; Krajewska and Powers, 1988; Attuquayefio and Buckle, 1987). The GC methods require some form of derivatization to form compounds that are sufficiently volatile for the analysis (Cooper et al., 1991). The extraction method reported here, eliminates the tedious chromatographic separation of the substances that interfere in the UV detection of capsaicinoids and provides the precision of direct spectrophotometric analysis.

2. MATERIAL AND METHODS

2.1. Apparatus

A Milton Roy Spectronic 3000 diode array spectrophotometer with 0.35 nm resolution, which was coupled to a Milton Roy 486 PC and User Data version 2.01 software for spectral data acquisition, storage and processing, was used.

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Commented [h3]: Scoville heat unit means measure of level of pungency or heat of a pepper or other substance. Give detail procedure which involves dilution with sugar-water solution.

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2.2. Reagents

All chemicals were analytical-reagent grade. Capsaicinoids (60% capsaicin, CAP) standard was obtained from Sigma Chemical Co. Stock CAP solution (10 mg⊕L^{-1}) was prepared by dissolving 100 mg in 100 ml of anhydrous ethanol (J.T. Baker). This solution was stable for at least one month in refrigeration. Buffer solution (1 mol·⊕ L^{-1}, pH 2.8) was prepared with chloroacetic acid (Aldrich) and adjusting to pH with NaOH solution. Adogen 464 (Aldrich) solution 1 g⊕L^{-1} in toluene (J.T. Baker) was used.

2.3. *Procedure*

To a series of 250 ml separating funnels, different volumes of CAP standard solution were added to cover the range from 4 to 44 µg·ml-1 of CAP. Suitable volumes of ethanol to get 2 ml, and 2.5 ml of buffer solution pH 2.8 were added to each funnel. The total volume was adjusted to 25 ml with water. Then, the solutions were extracted with portions of 10 ml adogen 464-toluene solution with vigorously shaken for 1 minute. The phases were allowed to separate accurately (López-de-Alba et al., 1997). The absorption spectra of organic phases were recorded from 200 to 350 nm against a blank extracted in the same way. The absorbance values were measurement at 286 nm and a calibration graph was obtained.

2.3.1. Determination in commercial and natural samples

Samples of Chili powdered and raw fruit. Amounts of 1.0 g were accurate weighed (by triplicate) and extracted with ethanol with continuous stirring for 15 min. The extracts were filtered, washed and collected into a 25 ml volumetric flask. The volume was made to the mark with absolute ethanol. Aliquots of 1-2 ml of the solutions were taken and treated in accord with the above-described procedure. Absorbance values were measurement to 286 nm and the concentration for CAP determined from the calibration graph.

Samples of 1.0 ml of sauce were suspended in ethanol and stirring for 15 min, filtered, washed, collected into 25 ml volumetric flasks and diluted to the mark with ethanol. Suitable aliquots of solutions were treated in accord with the above described procedure.

3. RESULTS AND DISCUSSION

Preliminary CAP extractions were carried out using isobutyl methyl ketone (IBMK) and toluene solutions of Adogen-464 and Aliquat-336. Better extraction yields were obtained with toluene, suggesting that the micelle assembly was better solvated in toluene than in IBMK with Adogen-464. The micelle formation was verified by extraction of CAP into toluene in the absence of Adogen-464; a spectrum with absorption maximum to 285 nm was obtained, but the intensity of this signal was ten times lower than the obtained in the presence of Adogen-464. In addition, the concentration of Adogen-464 selected was around ten times higher than the corresponding c.m.c.

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The effect of pH on formation and extraction of CAP-Adogen was studied in the range of 2.0 to 5.0. Better results were obtained to pH 2.8; to pH 3.5 sensibility decrease around 20% and to pH 4.0 and higher an emulsion was formed to make difficult the separation of phases. A pH of 2.8 was selected for further experiments and the best reproducibility and sensitivity were obtained with a chloroacetic acid-sodium chloroacetate buffer solution (1 mol· l-1). It was verified that shaking the mixture for 1 min was sufficient for quantitative extraction of the CAP. The volume ratio between the aqueous and organic phase on the extraction was studied and a VW: VO ratio of 2.5 was selected.

Possible interference of carotenes commonly present together to CAP in natural samples was studied. Extractions of carotenes-CAP mixture with toluene in presence and absence of Adogen-464 were made. Carotene was well extracted in toluene together CAP but in presence of Adogen-464 only the signal to 286 nm for CAP was obtained, making selective the proposed method. Because solubility of CAP, solutions are prepared in ethanol and a concentration of 8% in aqueous solution is necessary. An absorption spectrum of CAP-Adogen 464 micelle in toluene is shown in Figure 2. The analytical characteristics for the determination were evaluated using wavelength corresponding to the maximum absorption to 286 nm and the results obtained are given in Table 1.

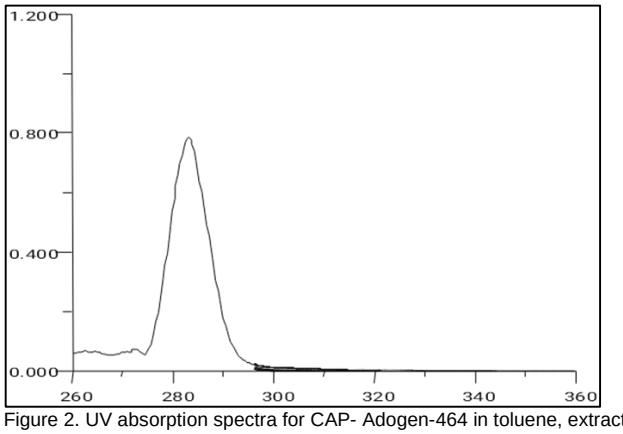

Figure 2. UV absorption spectra for CAP- Adogen-464 in toluene, extracted to pH 2.8.

Table 1. Analytical characteristics and statistical parameters for determination of CAP

λ_{max}	286 nm
Linearity range (µg ml^{-1})	0 - 44
Equation	$A = 0.0231C_{CAP} + 0.002$
Regression coefficient (r)	0.9990
dsr, (%) (40 µg ml^{-1})*	0.80
dsr, (%) (8 µg ml^{-1})*	2.12
E_{rel}, (%) (40 µg ml^{-1})*	0.74
E_{rel}, (%) (8 µg ml^{-1})*	1.52
L_d, µg ml^{-1} CAP**	0.259
L_Q, µg ml^{-1} CAP***	0.865

* (p = 0.05, n = 10)
** Detection limit = 3SB/m; SB = standard deviation of blank; m = slope of calibration
*** Quantification limit = 10SB/m; SB = standard deviation of blank; m = slope of calibration

The proposed method was applied to the determination of CAP in different commercial products and in the Capsicum fruits. The commercial products were sauces and chili powder samples added with lemon or salt, and the natural samples were green and red chili. The standard addition method was applied and the results obtained are presented in Table 2 and Figures 3. Table 3 shows the CAP content in the samples.

Table 2. Recovery results expressed as percentage (standard adition method)

Sample	CAP added (µg ml-1)	CAP found (µg ml-1)	Recovery (%)	
Spicy Sauce "maga"	4	4.15	103.75	
	8	8.39	104.88	
	12	12.54	104.50	103.95 + 0.89 %
	16	16.52	103.25	
	20	20.65	103.25	
	4	3.68	96.25	
	8	8.17	102.13	

Chili with salt and lemon McCormick	12	12.02	100.17	100.49 + 3.22 %
	16	16.47	102.94	
	20	20.19	100.95	
	4	4.35	108.75	
Chili powder Cal-Compack Foods	8	8.65	108.13	
	12	12.33	102.74	106.80 + 2.94 %
	16	17.17	107.33	
	20	21.41	107.05	
	4	3.95	98.75	
Chili powder American Food Ingr., Inc.	8	7.41	92.63	
	12	12.43	103.58	99.42 + 5.20 %
	16	16.28	101.75	
	20	20.08	100.40	
	4	4.05	101.25	
Serrano chili powder (green)	8	7.72	96.50	
	12	11.61	96.75	98.54 + 3.72%
	16	16.37	102.31	
	20	19.18	95.90	
	4	3.70	92.52	
Piquin chili Powder (red)	8	8.24	103.00	
	12	12.13	101.11	98.94 + 4.95%
	16	15.72	98.27	
	20	19.96	99.80	

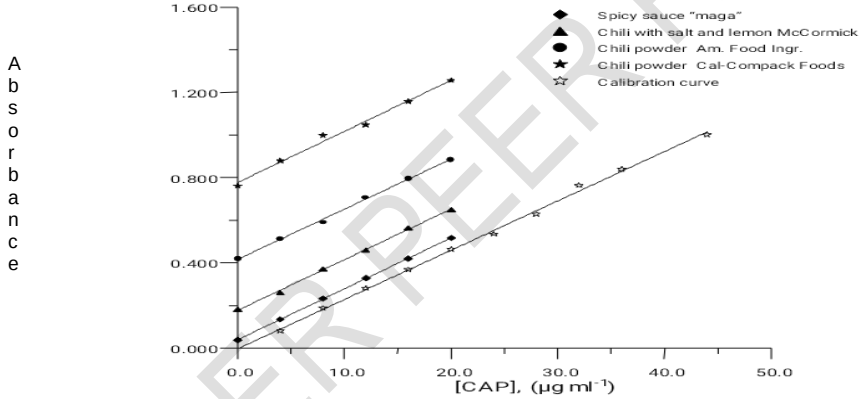

Figure 3. Typical standard addition curves in different commercial products.

Table 3. CAP content in commercial products Foods.

Sample	% Capsaicinoids
Spicy sauce "maga"	0.07 ± 0.001
Chili powder with salt and lemon McCormick	0.32 ± 0.02
Chili powder American Food Ingr., Inc.	0.75 ± 0.3
Chili powder Cal-Compack Foods	1.35 ± 0.05
Serrano Chili (green)	0.35 + 0.03
Cascabelillo Chili (green)	0.60 + 0.03
Piquin Chili (red)	0.82 + 0.03
Cascabel Chili (red)	1.36 + 0.05
Tree Chili (red)	1.40 + 0.03

4. CONCLUSION

The extraction of the CAP into toluene was achieved by micelle assembly formation with Adogen-464. Using this analytical procedure, a good analytical performance was obtained for the determination of CAP in different commercial food products. This extraction method is easy and quick.

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