Novel Approaches to Extraction Methods in Recovery of Capsaicin from Habanero Pepper (CNPH 15.192)

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ABSTRACT

Introduction: The objective of this study was to compare three capsaicin extraction methods: Shoxlet, Ultrasound-assisted Extraction (UAE), and Shaker-assisted Extraction (SAE) from Habanero pepper, CNPH 15.192. **Materials and Methods:** The different parameters evaluated were alcohol degree, time extraction, and solid–solvent ratio using response surface methodology (RSM). **Results:** The three parameters found significant ($p < 0.05$) were for UAE and solvent concentration and extraction time for SAE. The optimum conditions for the capsaicin UAE and SAE were similar 95% alcohol degree, 30 minutes and solid–liquid ratio 2 mg/mL. The Soxhlet increased the extraction in 10–25%; however, long extraction times (45 minutes) degraded 2% capsaicin. **Conclusion:** The extraction of capsaicin was influenced by extraction method and by the operating conditions chosen. The optimized conditions provided savings of time, solvent, and herbal material. Prudent choice of the extraction method is essential to ensure optimal yield of extract, thereby making the study relevant and the knowledge gained useful for further exploitation and application of this resource.

Keywords: *Extraction process*, factorial design, nutraceuticals, phytomedicines

SUMMARY

- *Habanero pepper,* line CNPH 15.192, possess capsaicin in higher levels when compared with others species
- Higher levels of ethanolic strength are more suitable to obtain a higher levels of capsaicin
- Box-Behnken design indicates to be useful to explore the best conditions of ultrasound assisted extraction of capsaicin.

Abbreviations used: Nomenclature UAE: Ultrasound-assisted Extraction; SAE: Shaker-assisted Extraction.

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DOI: 10.4103/0973-1296.210127

Access this article online
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INTRODUCTION

Capsicum spp is a consumed and appreciated for its pungency color, and flavor aroma,[1] also for the biological activities such as anti-inflammatory,[2] antioxidant,[3] and hypocholesterolemic[4]. The genus *Capsicum* comprises five domesticated species: *C. annuum, C. baccatum, C. chinense, C. frutescens,and C. pubescens.*[5] and contain different amounts of capsaicinoids, carotenoids, vitamins, and flavonoids.

Capsaicinoids are the principal secondary metabolites responsible for the pungency of *Capsicum* chili pepper fruit and biological activities of *Capsicum chili*[6-8]. This class of compounds possesses more than 50 capsaicinoids, which can be identified and quantified by HPLC, HPLC-MS, GC and GC-MS.[7]

Embrapa vegetable crops have an important *Capsicum* breeding program that has focused on the development of habanero-type cultivars (*C. chinense*, line CNPH 15.192).

This cultivar has the characteristic of having high level of pungency capsaicin, which makes it promising for use in foods and medicines. However, the development and efficient capsaicin extraction method for pharmaceutical and alimentary industry depends on method, herbal material, and optimization technique.[9]

The extraction method chosen must be fast, inexpensive, versatile and efficient and should have anease performance and no toxicity. The most widely used method for extracting capsaicin is based on extraction with hexane, which is very toxic and produces residual solvent.[10-12]

There are several techniques for process optimization, among them the simplex, univariate, and multivariate analyses associated with RSM.

Among these, multivariate analysis associated with RSM is efficient due to its versatility, ability to assess multiple factors, ease of performance, and robustness.[13-14]

Thus, the present study aimed to evaluate and optimize different capsaicin extraction methods, prioritizing operational versatility and low toxicity solvent extractors.

MATERIALS AND METHODS

Chemical and Reagents

The capsaicin standard used was purchased from USP Reference Standards (Philadelphia, PA, USA). All other chemical reagents were HPLC grade.

Plant Material

Habanero pepper, line CNPH 15.192, was provided by Embrapa Vegetables, Brasilia-DF. Mature fruits were cleaned and had the peduncle removed. They were then stabilized and dried in an oven with forced air circulation at

40°C for 48 hours. Dried fruit were ground in a knife mill TE-625 (Tecnal Ltda, Piracicaba, SP, Brazil) and the powder was stored at −4°C.

HPLC analysis

Capsaicin was identified and quantified by HPLC (Waters® .e2695) method. The separation method used C18 column, from Agilent Technologies® (USA) (250 mm × 4.6 mm i.d. × 5 μm particle size). Chromatographic system was performed with solvent acetonitrile, methanol, and water (5:3:2) at a flow rate of 1 mL/min. The separated capsaicin peak was identified by comparing the individual standard with the retention time and the UV 229 nm spectra. The methodology was validated in accordance to.[15]

Extraction of Capsaicin

UEA was performed in an ultrasonic cleaner bath (UNIQUE® USC 4800, 40 KHz) and SAE was carried out in a shaker (novatecnica, NT 155). The extractions were performed in volumetric flasks (5 mL) with different hydroethanolic mixtures.

Evaluation of Degradation of Capsaicin by UEA, SAE, and Soxhlet Extraction

A previous study of stability ($n = 3$) was done with standard of capsaicin solution (1.5 mg/mL), which was kept for 15, 30, 45, and 60 minutes in an ultrasound bath at 37°C (USC 1400, Unique®), shaker (Novatecnica®, NT 155) or Soxhlet at 90°C. A control solution (free extraction) in the same concentration was made and the areas of chemical marker were compared by HPLC.

Capsaicin Content

Two grams of dehydrated Habanero pepper fruit line CNPH(CNPH 15.192) pepper were placed in a Whatman 25 × 100 mm cellulose thimble. The extraction using Shoxlet at 90° C method ($n = 3$) was carried with 100 mL of ethanol 95% (vol/vol) with 5 cycles at 45 minutes each. The extract obtained was concentrated using a vacuum rotatory evaporator at 40°C. The dried extract was analyzed by HPLC.

Experimental design

UAE and SAE of the capsaicin contents of the *H. chili pepper* fruit were performed as described by the Box-Behnken, three factors, and three levels [Table 1]. The experimental runs were randomized to satisfy the statistical requirement of independence of observations. The second-order model employed for the response surface of capsaicin contents has the form:

(1) $$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum\sum \beta_{ij} x_i x_j$$

Where y is the capsaicin contents response variable, and (x^i) are the selected parameters to model the response. The parameters analyzed were extraction time (x^1), alcohol degree (x^2), and solid to solvent ratio (x^3). The various $\beta's$ represent the coefficients of the model. The two-factor interaction term β_{ij} represents either synergistic or antagonistic effects in the capsaicin extraction process. All the calculations were carried out using Design-Expert®version 7.0.0.[16]

To verify the predictive capability of the model, optimum conditions were established by RSM and comparisons between the predicted results and the practical values were carried out by experimental repetition using presumed optimal conditions. The Soxhlet extraction was performed with 2 mg/mL of herbal material by 30 minutes, with ethanol 95%.

Results and Discussion

The system suitability parameters were in accordance with the literature specifications [Table 2]. The HPLC method proved to be capable of

Table 1: Level of factors chosen for the trials

Factor	Low	Center	High
Extraction time (min, X_1)	10	20	30
Alcohol degree (vol/vol%, X_2)	35	65	95
Solid to solvent ratio (mg/mL, X_3)	2	4	6

Table 2: System suitability parameters values to capsaicin from H. pepper, line CNPH 15.192

Parameter	capsaicin	Recommendations
Repeatability [a]	<0.1%	RSD $\leq$ 1% to $n \geq$ 5
Tailing factor (T)	1.1	<2.0
Theoretical plates (N)	20000	>2000
Resolution	2.6	>2.0

[a]Data expressed as mean ± SD (standard deviation); *RSD % (relative standard deviation)*

Table 3: Validation parameters values obtained from HPLC-PDA method for the determination of capsaicin from H. pepper (CNPH 15.192)

Parameter		Capsaicin
Linearity	Linearity range (μg/mL)	0.5–40
Sensitivity	Limit of detection (*LOD*, μg/mL)	0.22
	Limit of quantification (*LOQ*, μg/mL)	0.34
Precision	RSD%	<0.1
Accuracy		
	Recovery 80%	100.22% ± 0.02 [a]
	Recovery 100%	100.99% ± 0.91 [a]
	Recovery 120%	100.00% ± 0.66 [a]
Robustness		
	Changing column mark/*RSD* (%)	
	Temperature of column/*RSD* (%)	<0.1
	Ratio of solvent/*RSD* (%)	

Data expressed as mean ± SD (standard deviation); RSD % (relative standard deviation)
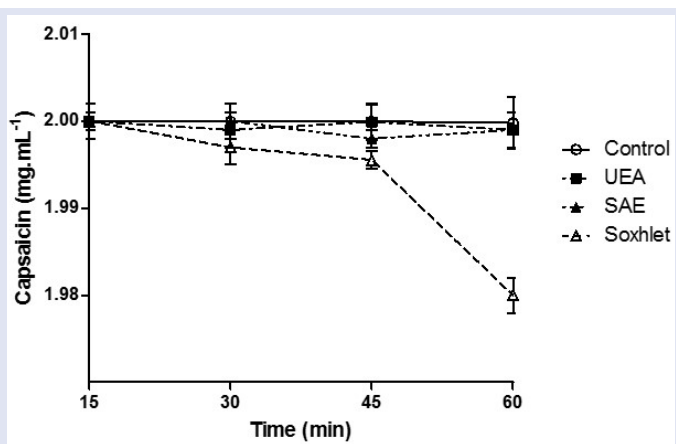

Figure 1: Evaluation of degradation of capsaicin by UEA, SAE and Soxhlet methods

providing data of acceptable quality, performing the selectivity test, linearity, precision, and accuracy. Table 3 highlights the values obtained from method validation; the calibration curves showed a linear response (0.5-40 µg/mL), obtaining correlation coefficients (r) 0.999. The LOD (0.22 µg/mL) and LOQ (0.34 µg/mL) showed that the present method is adequate and has the sensitivity to detect and quantify of capsaicin from *H. pepper* (CNPH 15.192).

The stability study showed that capsaicin content was not altered by the action of UEA or SEA [Figure 1]; there was a range of <0.25% between the sample content and the control. However, the extraction by Soxhlet degraded 2% of capsaicin after 45 minutes of extraction.

The total capsaicin content extracted by Soxhlet (exhaustion process) showed that *H. pepper* has 2.2% (22.0 mg/g) of capsaicin. The UEA recovered 90.7% (14.2–19.9 mg/g) and SAE 76.1% (8.3–16.9 mg/g) [Table 4].The higher extraction yield obtained by the Soxhlet is attributed to high extraction time, temperature, and drug solvent ratio. These factors

increase the solubility of capsaicin. This method demanded a long extraction time (225 minutes), a large volume of solvent (500 mL) and degraded the sample. Although UEA and SEA extracted less capsaicin (9.3–23.9% less) they did save 86% of extraction time and 99% of solvent.

The UEA recovered 15–41.5% more capsaicin than the SEA method [Table 4]. The higher extraction yield obtained by the UEA is attributed to the effects of acoustic cavitations in the solvent, which were produced by ultrasonic waves. The waves also exert a mechanical effect, increasing the penetration of the solvent into the herbal matrix and the contact surface between the solid and liquid phases.[17-19] The RSM showed that the $X1.X2.X3$, $X1.X2$ and $X2.X3$ had significant ($p < 0.05$) effect on UEA process and $X2$ and $X1.X2$ on SAE [Table 5]. Figures 2a, 2b, and 3 show that high level of extraction time ($X1$), alcohol degree ($X2$), and drug solvent ratio ($X3$) increase the extraction of capsaicin in both the extraction methods.

The optimal theoretical extraction parameters for capsaicin (2.0% to UAE and 1.70% to SEA) calculated by RSM were similar in both methods of extraction, 30 minutes of extraction time, 2 mg/mL of herbal material, and 95% of alcohol degree [Figure 2 and Figure 3].

Table 4: Box-Behnken 3³ design and observed responses

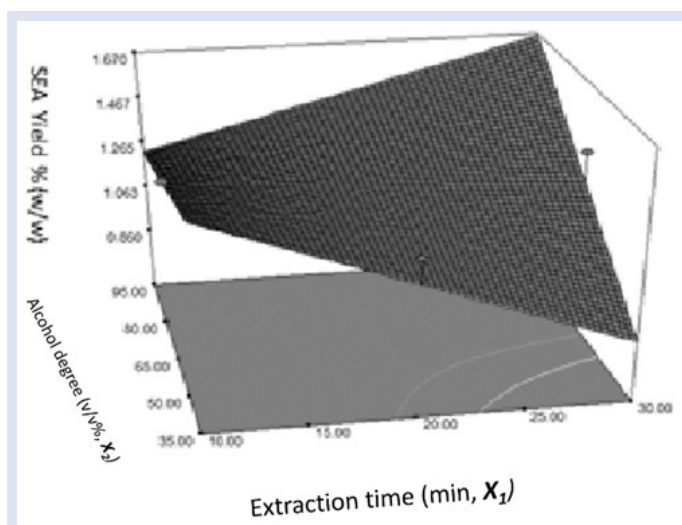| Run	X_1	X_2	X_3	UEA Yield %	SAE Yield %
1	20	35	6	1.402	1.279
2	20	65	4	1.602	1.273
3	20	65	4	1.639	1.265
4	20	65	4	1.641	1.346
5	10	65	6	1.493	1.364
6	30	65	6	1.426	1.387
7	30	35	4	1.432	0.834
8	20	65	6	1.616	1.339
9	20	65	4	1.627	1.271
10	30	95	4	1.997	1.695
11	30	65	2	1.851	1.505
12	20	95	6	1.615	1.415
13	20	35	2	1.786	1.511
14	10	65	2	1.708	1.492
16	20	95	2	1.651	1.501
17	10	35	4	1.510	1.583

Figure 2: Response-surface graphs representing the UEA effect on extraction of capsaicin.

Table 5: Summary of factors, effects and significances (p) ANOVA of UEA and SEA

	UEA				SEA			
Source	SQ	df	F-Value	p-value Prob > F	SQ	df	F-Value	p-value Prob > F
Model	0.344	6	11.31	0.0006	0.344	6	5.55	0.0090
X_1	0.030	1	6.02	0.0340	0.030	1	1.62	0.2312
X_2	0.050	1	9.90	0.0104	0.050	1	6.19	0.0321
X_3	0.140	1	27.57	0.0004	0.140	1	3.10	0.1084
$X_1 X_2$	0.082	1	16.23	0.0024	0.082	1	21.99	0.0009
$X_1 X_3$	0.011	1	2.17	0.1709	0.011	1	0.0023	0.9624
$X_2 X_3$	0.030	1	5.98	0.0344	0.030	1	0.419	0.5318
Residual	0.051	10			0.0508	10	0.0050	
Pure Error	0.0010	4	0.00026		0.0010	4	0.00026	

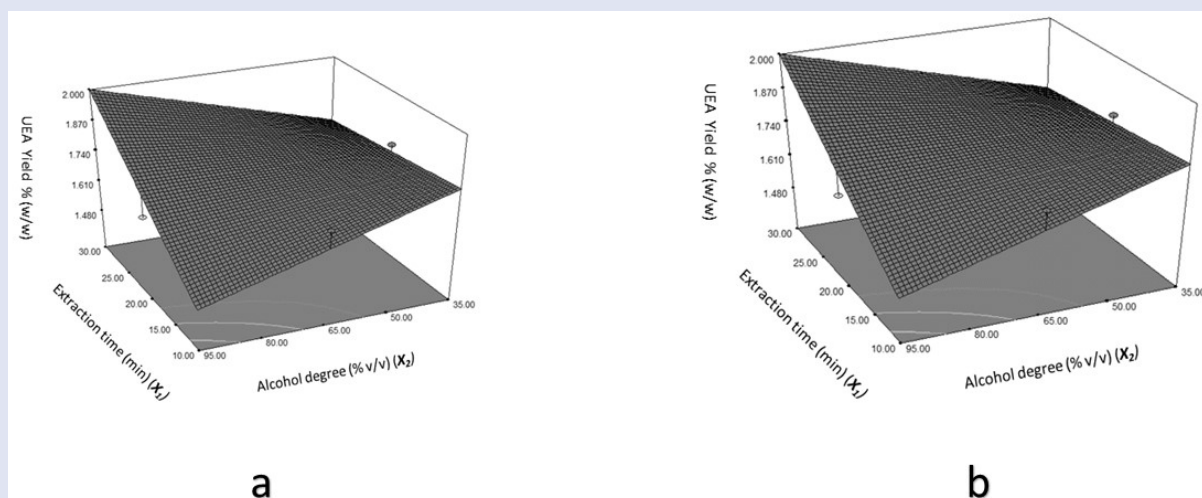*SQ: Sum of Squares; df :Degrees of freedom*

Figure 3: Response-surface graph representing the SEA effect on extraction of capsaicin from Habanero

Tests were conducted again in triplicate and showed that the capsaicin contents obtained from extraction under optimal conditions were 2.09 + 0.05% wt/wt ($n = 3$) to UEA and 1.71 ± 0.06% wt/wt ($n = 3$) to SEA. The good correlation between the theoretical results and the reexamined values confirmed that the response model represented the expected optimization well (Equation 1 and Equation 2, uncoded values).

(2) $\quad UAE\ yield\ \% = +2.3 - 0.014\,X_1 - 0.012\,X_2 - 0.1\,X_3 + 4.7 \times 10^{-4}\,X_1.X_2 + 1.45 \times 10^{-3}\,X_2.X_3$

(3) $\quad SAE\ yield\ \% = 2.71 - 0.16\,X_2 + 8.8 \times 10^{-4}\,X_1.X_2$

The choice of extraction method must be made carefully, as use of harmful solvents, high temperatures, and waste material need to be avoided. Currently, the use of harmful solvents in the manufacture of food and medicine has been strictly controlled by health agencies, including the FDA United States Food and Drug Administration (USFDA), the Brazilian Health Surveillance Agency (ANVISA), and the European Union Agency (EMA). With the increasing demand for drug and food, wastage of natural resources should be avoided. Advanced techniques have been used in optimization processes, helping to prevent wastage and increasing the quality.[20-21]

The UAE has many advantages, however, its use for extraction of bioactive compounds should be chosen carefully. During the formation of cavity bubbles, there is a momentary increase in temperature (550°C) and pressure (550 atm). This may accelerate the degradation of the compounds of interest, forming low molecular weight.[22-24]

CONCLUSION

The extraction of capsaicin was influenced by extraction method and by the operating conditions chosen. The optimized conditions provided saving of time, solvent, and herbal material. Prudent choice of the extraction method is essential to ensure optimal yield of extract; thereby making the study relevant and the knowledge gained useful for further exploitation and application of this resource.

Acknowledgements

Financial support and sponsorship

Conflict of Interest

None

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