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Evaluation of different solvent systems for the extraction and fractionation of oleoresins from guajillo peppers

Carlos Abel Amaya Guerra [1], *Sergio Román Othón Serna Saldivar, Enrique Cárdenas and Juan Antonio Nevero Muñoz*

Instituto Tecnológico y de Estudios Superiores de Monterrey. Programa de Graduados en Ingeniería de Alimentos. Monterrey, México

SUMMARY. Dried guajillo peppers were first extracted with four different solvents: ethanol, acetone, ethyl acetate and hexane with the aim of obtaining oleoresins which were further fractionated into red and paprika extracts. Results showed that as the polarity of the solvent increased the amount of pigments extracted also increased. Acetone had good affinity for pungent (capsaicin) compounds. Utilization of these solvents alone did not produce red and paprika oleoresins that meet commercial specifications. Fractionation of acetone extracted oleoresins with ethanol: water (90:10) yielded a precipitate and a solution. The precipitate and solution produced red and paprika extracts that meet pungency and color specifications. It was possible to obtain red and paprika oleoresins from mild guajillo peppers.

RESUMEN. Evaluación de diferentes solventes para la extracción y fraccionamiento de oleorresinas a partir de chile guajillo. Chiles guajillo deshidratados se sometieron a una extracción con cuatro diferentes solventes: etanol, acetona, etil acetato y hexano con el objetivo de obtener oleorresinas que fueron posteriormente fraccionadas en extractos rojo (picante) y de paprika (color). Los resultados muestran que conforme la polaridad del solvente aumentó, la cantidad de pigmentos extraídos también se incrementó. La acetona mostró una buena afinidad por los compuestos pungentes (capsaicina). La utilización de estos solventes solos no produjeron oleorresinas rojas y de paprika que cumplieran con las especificaciones comerciales. El fraccionamiento con etanol:agua (90:10) de las oleorresinas extraídas con acetona rindió un precipitado (extracto rojo) y una solución (paprika), que cumplieron con las especificaciones de pungencia y color, respectivamente. Fue posible obtener oleorresinas rojas y de paprika a partir de chiles guajillos con pungencia moderada.

INTRODUCTION

Peppers (*Capsicum annum*) are considered the second most important horticultural crop in Mexico and one of the most widely consumed by the population. *Per capita* consumption averages 6,4 kg/year (1), Heisser (2) mentioned that mesoamerican cultures cultivated peppers between 5.200-3.400 years B.C., making this crop one of the oldest of the Americas. There are many wild and cultivated strains within the *Capsicum annum* species, being the most important ones: jalapeño, ancho, serrano and guajillo. Guajillo pepper, also known as rattle pepper, has a rich and distinctive purple red coloration, vary in pungency and are usually sun dried before marketed. It is preferred by farmers due to its high productivity when compared with other cultivars (3). There are three types of oleoresins produced commercially: capsicum, red pepper and paprika. The capsicum oleoresin is usually obtained from pungent peppers and is treated to remove pigments and other compounds so to concentrate capsaicins and obtain an extract with at least 500.000 Scoville units and a maximum of 4.000 ASTA color units. Peppers most frecuently used are African peppers (*Capsicum frutescens* L.) The red oleoresin is an intermediate extract with a minimum of 100.000 Scoville units and a maximum of 20.000 ASTA color units while paprika oleoresins should contain no more than 4.000 Scoville units and more than 40.000 ASTTA color units. *Capsicum annum* L. pods are most commonly used to obtain paprika (4).

Pungency of peppers is mainly due to the quantity of vanillinamides which include capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin (5). More than 90% of the pungency of peppers is due to capsaicin and dihydrocapsaicin. Compounds responsible for imparting color are pigments such as capsanthin, capsorubin and cryptocapsin (6).

The objective of this research was to obtain two different oleoresins (red pepper and paprika type) that meet commercial specifications by solvent extractions from mild guajillo peppers.

MATERIAL AND METHODS

Raw material: Guajillo peppers (Real Mirasol variety) grown and harvested during the 1993 June-September cycle were obtained from a commercial farm located near Matehuala, San Luis Potosí, México. Pods were artificially dehydrated at the production site in a tunnel air drier. Twenty kilograms of peppers with 6,4% moisture were stored in sealed polyethylene bags under refrigeration (4 °C), Upon one day equilibration at room temperature, peppers were ground in a hammer mill equipped with a 2,5 mm screen.

Pepper analysis: Ground guajillo peppers were characterized for proximate composition using standard procedures (7) and microbiologically assayed for total plate count and yeast/molds using ICMSF (8) procedures. Particle size distribution of the ground material was performed using a Ro-Tap equipped with US N° 12, 50, 79, 80 and 100 mesh sieves. One hundred gram samples were placed on top of the US N° 12 sieve, shaken for 15 min and then fractions recuperated and weighed. Pepper pungency was determined by

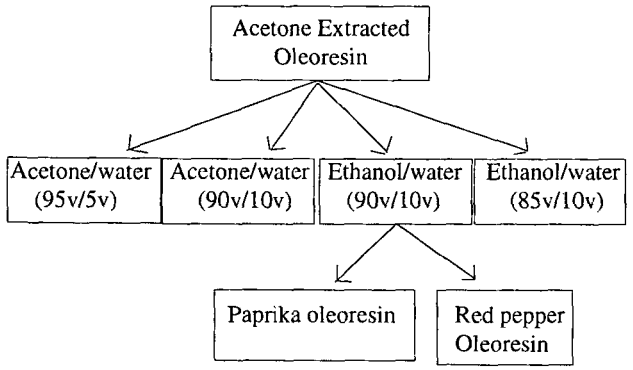
1 Professor, Universidad de Monteerrey. México

method 21,1 of ASTA (9) using an HPLC system (Hewlett Packard 1050) equipped with an UV detector set at 280 nm. Capsaicin and homocapsaicin were separated from pigments in a C18, 4,6 mm x 250 mm tubular column. Color, expressed as ASTA units, was determined following ASTA (9) method 20,1. Pepper pigments were extracted with acetone, then absorbance was determined at 460 nm with a spectrophotometer (Coleman Mod. 6/20).

Oleoresin production: Extraction. One hundred gram samples were placed in a thimble of a Soxhlet laboratory extraction apparatus. Samples were extracted with hexane, ethyl acetate, acetone and ethanol. Extraction time was completed when recirculant solvents did not have any visual coloration. In most instances, three hours were required to fully extract oleoresin from samples. Then, solvents were recuperated in a Rotary Evaporator operating at the evaporation temperature of each solvent. Solvent leftovers were removed by subjecting the desolventized extract to vaccum drying (15 psi) at 65 °C for 2 hr.

Fractionation. Preliminary studies indicated that acetone extracted oleoresin contained the highest amount of pungent compounds and a reasonable color value. Therefore, it was selected as the raw material for further fractionation. Fig. 1 shows the solvent: water systems that yielded two distinctive fractions and had the potential to obtain red and paprika oleoresins. The acetone extracted oleoresin was mixed with 7 parts of the solvent system, agitated for 1 hr at room temperature and then passed through a separation funnel to obtain a precipitate (paprika) and a solution (red oleoresin).

FIGURE 1
Fractionation scheme utilized to obtain paprika and red oleoresins from guajillo peppers



Oleoresin analysis: Oleoresin pungency and color were determined following the official methods described above (9).

Statistical analysis: Oleoresin extractions were run in triplicate. Oleoresin yield, pungency and color were analyzed following ANOVA procedures using a complete randomized design. Treatments means were compared using Tukey's test at a level of significance of 5%. Statistical analysis was run using the Statistical Analysis System package (10).

RESULTS AND DISCUSSION

Table 1 shows the chemical composition and microbiological counts of ground-dried guajillo pepper. Proximate composition values are within ranges reported by Quintin (11). Total plate and yeast/molds counts were below standards (12) for total plate counts 300.000 CFU/g and for yeast/molds 6.000 CFU/g. This is important because Purseglove (4) reported degradation of capsaicin and fatty acids by *Aspergillus niger* and *A. oryzae* resulting in reduced yield and quality of oleoresins. The guajillo pepper had around 10.000 Scoville units indicanting mild pungency. The dried pepper had approximately 3.500 ASTA color units (Table 1).

TABLE 1
Chemical composition and microbial counts of the guajillo pepper[a]

Moisture, %	6,4
Crude protein (N x 6.25 %)	13,4
Ether extract, %	10,0
NFE[b], %	45,7
Crude fiber, %	21,1
Ash, %	3,4
Pungency, Scoville units	10.474,7
Color, ASTA units	3.504.1
Total plate count, CFU/g[c]	85.000
Yeast and molds, CFU/g[c]	4.000

[a] Composition values are expressed «as is» basis.
[b] Nitrogen free extract. Calculated by difference, 100- Moisture-Protein - Crude Fiber- Ether Extract- Ash.
[c] Colony forming units per gram.

The particle size distribution of the dried/ground pepper were as follows: +12=0,1%, +50=3,7%, +70=86,1%, +80=7,3% and +100=2,8%. More than 85% of the particles were retained by US N° 70 mesh sieve. According to Mathew (13) the finer the particle the better the efficiency of oleoresin extraction.

Yields, pungencies and colors of oleoresins extracted with four different solvents is presented in Tables 2 and 3. Results show that the ethanol extracted the highest amount of oleoresin followed by acetone and ethyl acetate and hexane. Interestingly, as the polarity of the solvent increased, oleoresin yield also increased. Acetone extracted the highest amount of pungent compounds followed by ethanol extracted oleoresin. Hoffman et al (14) mentioned that acetone has high affinity for capsaicinoids. The relative extraction efficiency was 85,4% and 82,0% for these solvents, respectively. Ethyl acetate and hexane extracted less than 50% of the pungent compounds (Table 2).

TABLE 2
Yield and pungency of the guajillo pepper extracts

	Oleoresin Yield (g/100 g)	Oleoresin Pungency (Scoville units)	Total Pungency (Scoville units)	Relative Efficiency of Pungency Extraction (%)
Pepper	—	—	10,474.7[a]	100,0
Oleoresins				
Ethanol	11.8[a]	72,790.5[b]	8,589.3[b]	82.0
Acetone	10.4[b]	85,979.3[a]	8,941.8[b]	85.4
Ethyl acetate	8.1[c]	54,167.8[c]	4,387.6[c]	41.9
Hexane	8.4[c]	58,943.8[c]	4,951.3[c]	47.2

a-c Means within the same column with the same superscript are not significantly different (p>0.05).

TABLE 3
Yield and color of the guajillo pepper extracts

	Oleoresin Yield (g/100 g)	Oleoresin Color (ASTA units)	Total Color (ASTA units)	Relative Efficiency of Color Extraction (%)
Pepper	—	—	3,504.1[a]	100.0
Oleoresins				
Ethanol	11.8[a]	22,030[b]	2,599.5[c]	74.2
Acetone	10.4[b]	33.414[a]	3,475.1[a]	99.2
Ethyl acetate	8.1[c]	34,909[a]	2,827.6[b]	80.7
Hexane	8.4	41,350[a]	3,473.4[a]	99.1

a-c Means within the same column with the same superscript are not significantly different (p>0.05).

Acetone and hexane extracted the highest amount of color compounds followed by ethyl acetate and ethanol. The relative extraction efficiency of color compounds for acetone and hexane was higher than 99%. The ethyl acetate and ethanol were the least efficient in extracting color (Table 3). After this study it was concluded that the acetone was the best solvent for extracting both pungent and color compounds, according to Attuquayefio & Buckle (15) acetone gave the highest yields of capsaicins and also extracted significant levels of pigments, therefore it was the solvent system selected to perform further fractionation. This is because the acetone oleoresin did not meet commercial standard specifications (4) for red pepper oleoresin (minimum 100.000 Scoville units) and paprika oleoresin (minimum 40.000 ASTA color units).

Acetone and ethanol mixed with water were selected as the fractionation solvent system. Acetone was selected due to its high affinity for pungent compounds whereas ethanol due to its low efficiency for extracting color compunds (is a normal industrial procedure reextract oleoresin with ethanol for concentrated the capsaicinoids compounds). Preliminary studies done at different acetone: water and ethanol: water ratios indicated that addition of more than 10% and 15% water to acetone and ethanol did not produce two distinctive fractions (a precipitate and a solution) at room temperature, therefore trials were run using 5 and 10% water for the acetone and 10 and 15% water for the ethanol. Table 4 shows yields, pungency and color of the solution fraction. The 90:10 acetone water ratio yields, pungency and color of the solution fraction. The 90:10 acetone water ratio yielded the highest amount of solution, but contained the lowest amount of pungent compounds and the highest amount of color (Table 4). Acetone yielded extracts that meet the specification for red pepper oleoresin (>100.000 Scoville units), but exceded the minimum standard for color (<20.000 ASTA units). The ethanol solvent systems were the only ones that meet both pungency and color standards for red pepper oleoresin.

Table 5 shows yields, pungency and color of the precipitate fraction. The ethanol solvent systems yielded the highest amount of precipitate but only the 90:10 ethanol/water precipitate met specifications for a paprika type oleoresin (>40.000 ASTA color units and <4.000 Scoville units). The unique solvent system that produced a red pepper and paprika oleoresins that met minimum requirements was 90:10 ethanol/water. The solution fraction (Table 4) yielded 33,2% of a red pepper oleoresin which contained almost 200.000 Scoville units and approximately 2.300 ASTA color units and <4.000 Scoville units). The unique solvent system that produced a red pepper and paprika oleoresins that met minimum requirements was 90:10 ethanol/water. The solution fraction (Table 4) yielded 33,2% of a red pepper oleoresin which contained almost 200.000 Scoville units and approximately 2.300 ASTA color units and 66,8% of a precipitate fraction with 2.554 Scoville units and approximately one and a half times the minimum color units for a paprika oleoresin.

Results of this study demonstrated that fractionation is an alternative to obtain oleoresins from peppers that have mild pungency and color.

TABLE 4
Yield, pungency and color of the oleoresin in solution

Solvent/water ratio (v/v)	Solution Oleoresin Yield	Pungency (Scoville Units)	Color (ASTA Units)
Acetone 95/5	46.7[b]	178,268.2[a]	22,009[a]
Acetone 90/10	72.9[a]	112,386.3[b]	25,050[a]
Ethanol 90/10	33.2[c]	194,661.7[a]	2,303[b]
Ethanol 85/15	42.1[b]	170,949.6[a]	4,170[b]

a-c Means within the same column with the same superscript are not significantly different (p>0.05).

TABLE 5
Yield, pungency and color of the oleoresin precipitate

Solvent/water ratio (v/v)	Precipitate Oleoresin Yield	Pungency (Scoville Units)	Color (ASTA Units)
Acetone 95/5	53.3[b]	4,935.7[b]	21,405[c]
Acetone 90/10	27.2[c]	17,698.0[a]	15,005[a]
Ethanol 90/10	66.8[a]	2,554.2[b]	56,711[a]
Ethanol 85/15	57.9[b]	2,476.4[a]	42,872[b]

a-c Means within the same column with the same superscript are not significantly different (p>0.05).

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