

COMMUNICATIONS

A High-Yield Method for the Extraction and Purification of Capsaicin

Capsaicin can be extracted from lyophilized fresh green chili peppers using modifications of previously reported procedures. When used to comparatively extract dried red peppers, a 400% greater yield was obtained using the described method. No chlorinated hydrocarbon solvents are utilized in this procedure, thereby complying with preliminary standards published by the National Cancer Institute.

Workers have utilized various methods for the isolation of the pungent flavoring agent, capsaicin, from the dried ripe fruit of *Capsicum frutescens* (Nelson, 1910; Nelson, 1919; Tire, 1933; Jordan et al., 1942; Todd, 1958; Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry on Methods of Assay of Crude Drugs, 1959, 1964; Karawya et al., 1967; Hartman, 1970). These procedures involved the extraction of an oleoresin from the ground, dried peppers using organic solvents, removal of the solvent, and separation of the capsaicin from the oleoresin mixture. In an attempt to increase the yield and the purity of the isolated capsaicin, we endeavored to utilize fresh green peppers as the source of the active principal and to modify existing methods of extraction.

EXPERIMENTAL SECTIONS

Reagents. The following reagents were used: saturated barium chloride; saturated alcoholic silver nitrate; ligroine (petroleum ether, boiling range 60–110 °C); capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), obtained from Sigma Chemical Co., St Louis, Mo. Capsaicin was used as a reference standard for comparison of absorption spectra and was used in the determination of recovery values through the various stages of the procedure.

Procedure. Four kilograms of fresh ripe jalapeno peppers were pulped in a Waring blender without the addition of any liquid. The resultant mash was lyophilized to complete dryness and stored at -30 °C until use. One hundred gram portions of the dried material were extracted by agitation for 2 h in 1.5 L of acetone and allowed to stand until the particulate matter settled. The acetone layer was decanted and stored. This process was repeated until the acetone was nearly colorless (3–4×). The insoluble residue was filtered to dryness using a Buchner funnel under vacuum. The resultant residue was a finely divided cream-colored powder.

The decanted acetone extracts and the filtrate were pooled and concentrated under vacuum to 150 mL. This solution was dark green due to the presence of extracted chlorophylls.

The concentrated extract (oleoresin) was diluted with 4 volumes of deionized water, the pH adjusted to 11.0 with 0.5 N KOH, and allowed to stand 12 h at ambient temperature. Fifty milliliters of this solution was further diluted with 1.5 volumes of deionized water and the pH readjusted to 7.5 with concentrated HCl, following which it was extracted three times with diethyl ether. Phase separation was conducted in a separatory funnel, and the ether extracts were combined. The ether was distilled under vacuum and the resultant brownish-green pungent oily residue was dissolved in 90% methanol made slightly

alkaline with alcoholic KOH. From this point the procedure followed that of Nelson (1910), beginning with the addition of a saturated aqueous solution of barium chloride. Final recrystallization and decolorization was conducted by reflux in ligroine.

This method is summarized as a flow diagram in Figure 1. Analyses for purity were conducted using ultraviolet and infrared spectrophotometric comparisons.

RESULTS AND DISCUSSION

Prior to resorting to the lengthy procedure of Nelson (1910), attempts were made to extract capsaicin from portions of the oily residue of both fresh green peppers and dried red New Mexico hot peppers using the methods of Jordan (1942), Todd (1958), and Hartman (1970). None of these methods yielded compounds of high enough purity due to the presence of tissue chlorophylls. These methods recommended the use of activated charcoal (e.g., Norit A, Norit AG-11, or Darco G-60) for the removal of colorings and impurities. Small amounts of the varying types of charcoal were added to solutions of Sigma capsaicin in methanol. The suspensions were mixed by stirring for 10 min and separated by filtration. The methanolic solutions were assayed by the methods given in *Analyst (London)* (Joint Committee of the Pharmaceutical Society, 1959, 1964). At least 60% of the added capsaicin was found to be adsorbed by the charcoal (alkaline Norit A, 100% adsorption, Darco G-60, 61% adsorption). There was no increase in purity, resolution, or yield of capsaicin when acid-washed charcoal was used.

The spectrum of the capsaicin, obtained by the Hartman (1970) and Todd (1958, 1961) methods, when dissolved in 0.02 N NaOH in 80% MeOH and in 0.01 N HCl in 80% MeOH were not the same as those seen in *Analyst (London)* (Joint Committee of the Pharmaceutical Society, 1959) nor that of the Sigma capsaicin. The peak at 280 nm obtained using the Hartman and Todd procedures was considerably broadened, thereby masking the 240–250 nm peak seen with preparations in which chlorophyll contaminants had been removed.

A known amount of pure capsaicin dissolved in acetone and water, when processed through the Nelson procedure, showed no change in the absorption spectrum when compared with the starting material, indicating that this procedure will not degrade the molecule.

The ultraviolet spectra of capsaicin extracted from green jalapeno peppers prior to and following the final recrystallization from ligroine are identical with the spectra of the Sigma compound and those shown in *Analyst (London)* (Joint Committee of the Pharmaceutical Society, 1959). When a small quantity of the extracted crystals were dissolved in MeOH and the absorbance measured at

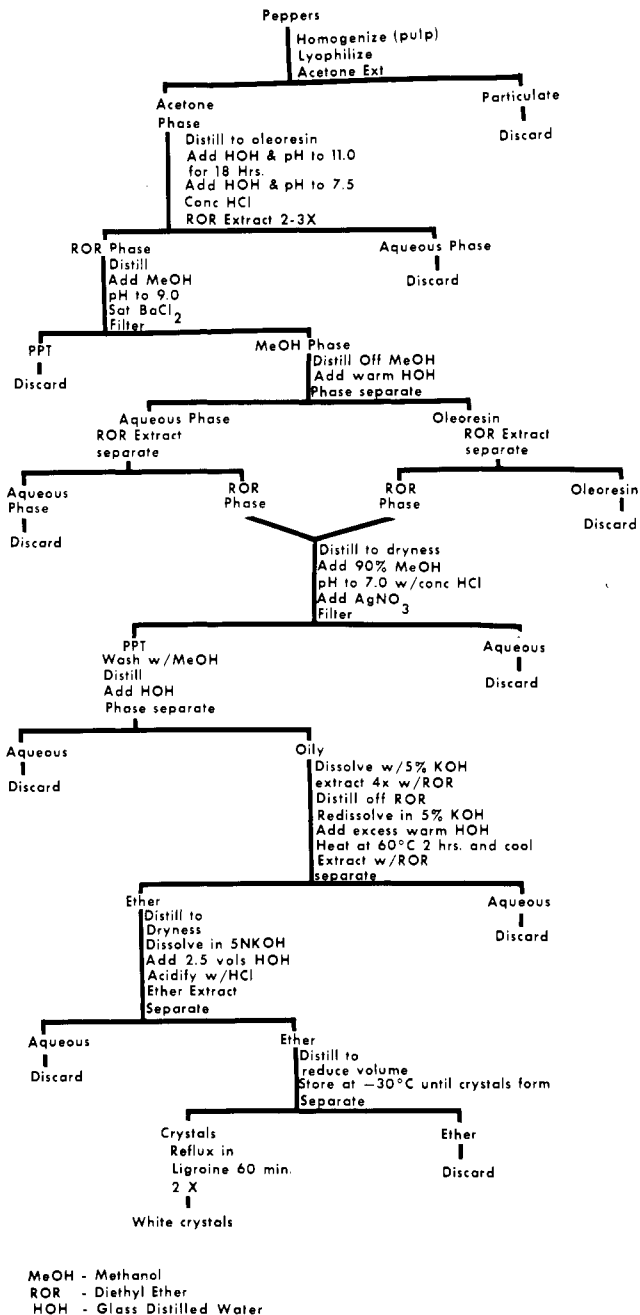


Figure 1. Flow diagram for extraction of capsaicin from green chili peppers.

270, 280, and 290 nm, the ratios of 270/280 nm and 290/280 nm were 0.601 and 0.549, respectively, which compare closely with those of the pure compound given in *Analyst (London)* (Joint Committee of the Pharmaceutical Society, 1959); 270/280 nm = 0.60:290/280 nm = 0.53.

Previous reports of extractions using dried red peppers as the source of this compound have resulted in yields in the range of 0.142% (Nelson, 1910) to 0.239% of the dry weight of the peppers extracted (Hartman, 1970). Using the above described procedures, beginning with fresh green peppers, the original 4 kg of peppers yielded 480 g of dried material following lyophilization. Processing this material resulted in the extraction and purification of 5.375 g of

capsaicin, for a yield of 1.12%. The reproducibility of this method is 1.120 ± 0.037 g/100 g of dried peppers. This represents an average 5.8X increase in yield when expressed as grams of capsaicin extracted/100 g of dried green pepper material. When this procedure was used to extract dried New Mexico hot red peppers, a yield of capsaicin equal to 0.562% of the dry weight of the peppers was obtained. The capsaicin extracted from the Mexican green Jalapeno peppers is identical with that contained in the various species of hot red peppers. The infrared spectrum of the extracted capsaicin from green peppers is identical with that obtained from the Sigma compound and those characteristic frequencies seen in the studies of Muller-Stock et al. (1973).

Because of properties of capsaicin, a mandatory consideration in the extraction process is that all distillations must be conducted under a vacuum of at least 4×10^{-5} Torr to prevent degradation of the compound.

At the start of this project only fresh green varieties of peppers were available for use. Future work will attempt to utilize fresh red peppers as a source of capsaicin. It is hoped that intrinsic problems involved in the removal of chlorophylls will be minimized without affecting the ultimate yield of capsaicin. Additionally, during the course of this research, the National Cancer Institute issued a preliminary report implicating some chlorinated hydrocarbons used as solvents in extracting oleoresins from flavoring species as causes of teratogenesis (*Chem. Eng. News*, 1975). Action on this finding may result in the classical methods of extraction and the Hungarian Patent 157919 (1971) becoming obsolete.

CONCLUSION

Capsaicin can be extracted from fresh green chili peppers in yield substantially greater than previously reported methods. The procedure for conducting this extraction, moreover, is in complete compliance with indications of preliminary standards set forth by the National Cancer Institute.

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