pounds of this series and a well-characterized odor of bell pepper. Surprisingly, we found that the L isomer had a different odor and a much higher threshold. It follows that, if the compound obtained from commercial menthone is free from cis isomers, the bell pepper odor is due only to the enantiomer D-*trans*-4-isopropyl-7-methylcyclohexathiazole; its olfactory threshold then should be 0.18 ppb, more than 100 times lower than that of its enantiomer. This finding, however, needs further investigation. A comparison between the structure of 4-isopropyl-7-methylthiazole and that of 4-isobutyl-5-isopropylthiazole (Figure 2b), which shows an extremely intense odor of bell pepper (Pelosi and Pasqualetto, 1981), shows that the first compound mimics a particular situation among the many conformations of the second derivative and defines with greater accuracy the shape requirements for the hydrophobic part of such odorants, and, consequently, the complementary shape of the corresponding olfactory receptor. We plan to measure binding constants between these odorants and the specific olfactory receptor and to establish correlations between such constants and odor properties.

Registry No. I, 57246-59-0; II, 5661-10-9; III, 4433-49-2; IV, 7140-68-3; V, 84648-03-3; VI, 84648-04-4; (±)-VII, 84648-05-5; (±)-VIII, 84648-06-6; (±)-IX, 84648-07-7; DL-*trans*-X, 84648-08-8; L-*trans*-X, 84710-13-4; D-*trans*-X, 84710-14-5; L-menthone, 14073-97-3; 2-bromocyclooctanone, 39261-18-2.

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Capsaicin Production in Sweet Bell and Pungent Jalapeno Peppers

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Morphological, histological, and chemical analyses of the interlocular septums of jalapeno peppers and bell peppers were compared. Scanning electron, bright-field, and transmission electron microscopic examinations of the interlocular septums showed little histological difference of the interlocular septa. Glandular regions of oil-producing cells are present in both varieties of peppers. Thin-layer chromatography of the oil produced by pepper gland cells demonstrated that bell peppers produce neutral lipids, glycolipids, and capsaicinoids. Conclusive evidence was found that capsaicin is synthesized in the glandular areas of the interlocular septum of jalapeno peppers.

The jalapeno pepper originated in Jalapa, Mexico, and is becoming increasingly popular with U.S. consumers. One of the more desirable characteristics of jalapeno peppers from a consumer's point of view is pungency, or the ability to produce an organoleptic sensation of heat. The component of jalapeno and other peppers which produces this sensation is capsaicin, a chemical compound that is odorless and flavorless. It is located mainly in the cross walls of hot peppers and spreads throughout the pod during processing. The outer wall of the raw jalapeno has a flavor identical with that of bell pepper (Huffman et al., 1978).

The location of synthesis of capsaicin within the fruit is a subject of much dispute. Newman (1953) felt that the seeds contained little or no capsaicin. Balbaa et al. (1968) published data that indicated the opposite. Both, however, demonstrated that the majority of capsaicin was associated with the dissepiment portion of the fruit. Huffman et al. (1978) confirmed this using gas chromatography. They determined that higher levels of capsaicin are found in the cross walls. Small amounts found in the seed portion was thought to be due to surface contamination during dissection. They examined the cross wall portion of the jalapeno with light microscopy and failed to detect the presence of anatomical structures associated with the capsaicin. However, an intense yellow pigmentation can be observed in both hot and sweet peppers.

Plant glands are a distinct group of highly specialized cells. Glands are composed of secretory cells and often different kinds of auxiliary cells. Different terms are used to denote the type of secretion process. If the secreted material passes directly through the plasmalemma, the process is called eccrine. Material that is transported across the membrane, collected in vesicles, and extruded by exocytosis is called granulocrine secretion. Both forms of secretion are said to be the merocrine type (Schnepf, 1974).

The jalapeno pepper secretes an essential oil that is commonly known as capsaicin, but the type of gland or cell that produces it was unknown prior to this study. the presence of specialized cells which synthesize capsaicin in the cross walls was delineated as well as other aspects of the production of essential oils in jalapenos. Histological

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and morphological differences in the interlocular septae of sweet and pungent peppers were related to lipid content and composition and also capsaicin content in sweet and pungent peppers. Organoleptic pungency was also related to capsaicin and lipid content. As a result of this study, simplified methods of quantitating capsaicin based upon the lipid content of the peppers can possibly be developed.

EXPERIMENTAL SECTION

Raw Product. Jalapeno peppers were grown at the Texas A&M University research station in Weslaco, TX. The were harvested at the green ripe stage and shipped to College Station for analysis.

Capsaicin Analysis of Dried Peppers. Four cultivars of peppers—three hot (JM, TMJ, T85) and one mild (TAM)—were cut into sections and dried in a vacuum oven at 50 °C and 30 mmHg for 12 h until a constant weight was achieved. The peppers were ground in a Wiley mill to pass a No. 30 USAA screen.

Capsaicin analysis was completed according to the methods of Huffman et al. (1978) using a Varian Model 2700 gas chromatograph. Calculations for total capsaicin followed the method of Todd et al. (1975).

Capsaicin Analysis of Fresh Peppers. Fresh jalapeno peppers were dissected and interlocular septums were removed. A sharp probe was used to rupture the cuticle along the septums carefully avoiding penetrating the cells, while the tissue was viewed with a compound dissecting microscope at a magnification of 10×. The areas were washed with drops of chloroform and extract was collected in a small beaker. The extract was evaporated to dryness in a flash evaporator at 50 °C and rehydrated with THF with 1 mg/mL octacosane as an internal standard. The extract was silylated with MSTFA and injected into a Varian 2700 gas chromatograph with a temperature program of 170 to 230 °C rising at 4 °C/min.

Total Lipids of Interlocular Septum. The total lipids of the interlocular septums of bell peppers and of the four cultivars of jalapeno peppers grown at Weslaco, TX, were analyzed by using the method of Folch et al. (1957). Five grams of whole cross wall tissue was blended with a 2:1 (v/v) mixture of redistilled chloroform methanol with a solvent:tissue ratio of 20:1. The extract was filtered through Whatman No. 52 filter paper and given a wash with 0.05 N aqueous KCl (Folch et al., 1957).

Thin-Layer Chromatography. The contents of the blisters of jalapeno and bell pepers were analyzed by using thin-layer chromatography. A sharp probe was used to rupture the blisters on the interlocular septae of the peppers while they were viewed with a compound microscope at 10×. The contents of the blisters were carefully scraped out, weighed, and dissolved in 1 mL of chloroform. Thin-layer chromatography was performed on glass plates coated with silica gel in a layer 0.25 mm thick. The extract was spotted on the plates, and the plates were developed in a solvent system of diisobutyl ketone–acetic acid–water (40:25:2 v/v) (Kates, 1972). Lipid classes were identified by staining with Rhodamine G6 and from comparison with the data of Kates (1972).

Ether Extraction. An ether extraction of ground dried whole peppers was performed by using the method of Lee et al. (1975). The extracts were filtered through Whatman 52-grade filter paper, evaporated to dryness in a flash evaporator, and weighed.

Glutaraldehyde and Osmium Fixation. The interlocular septums of bell and jalapeno peppers were removed and the glandular areas were sectioned into 1-mm^2 pieces. The samples were placed immediately into a fixative of 3% glutaraldehyde and 3% paraformaldehyde in a 0.05 M Pipes buffer at a pH of 7.4 and at 4 °C. The sections were held in the fixative for 5 h. They were rinsed with cold 0.05 M Pipes buffer for 1 h with three to five changes and were placed in 1% osmium tetraoxide solution buffered with 0.05 Pipes buffer and held for 5 h at 4 °C. The tissues were rinsed with four to five changes of cold distilled H_2O for a period of 1 h, then placed in aqueous 2% uranyl acetate, and held at 4 °C overnight. They were rinsed with three to five changes of distilled H_2O and dehydrated in a series of alcohol solutions at concentrations of 25% C_2H_5OH, 70% C_2H_5OH, 100% C_2H_5OH (3×), and then 100% acetate (2×). They were held in each alcohol concentration for 30 min and each change of acetone for 20 min. The fixed specimens were placed in an acetone–resin mixture (50:50 v/v) for 24 h. The mixture was changed to acetone–resin (25:75 v/v) for another 24 h and then finally to a 100% resin for 24 h. The specimens were embedded in 100% Spurr's resin (Spurr, 1969).

Bright-Field Microscopy. The embedded specimens were mounted on wooden stubs, and sections of 1 μm thickness were obtained by using a Sorvall MT-2B ultramicrotome and stained with azure B and methylene blue (1:1 v/v). The sections were examined with a Wild M-20 compound microscope. Micrographs were recorded with Kodak panatomic X film with an ASA of 32.

Transmission Electron Microscopy. The embedded specimens were sectioned to a thickness of 600–700 Å using a Sorvall MT-2B ultramicrotome and placed on 300 × 75 mesh grids. They were stained with uranyl acetate and contrasted with lead citrate. The sections were examined using a Philips 300 transmission electron microscope. Information was recorded on orthochromatic negative film at a KV of 60.

Scanning Electron Microscopy. The specimens to be used for scanning electron microscopy were fixed in the same manner as for transmission electron microscopy. They were attached to aluminum stubs with silver conductive paint and coated with gold palladium. The mounted specimens were examined with a Cambridge Model S-4 stereoscan electron microscope. Micrographs were recorded from the cathode ray tube with Polaroid 55 positive–negative film.

$KMnO_4$ Fixation. Tissues from cross walls of bell and jalapeno peppers were fixed with a 4 °C aqueous $KMnO_4$ solution for 2 h. The samples were rinsed with 4 °C distilled H_2O until no more $KMnO_2$ could be rinsed out. They were dehydrated in an alcohol series in the same manner as the tissues fixed in glutaraldehyde and embedded in Spurr's low viscosity resin (Spurr, 1969). One micrometer thick sections were obtained by using a Sorvall MT-2B ultramicrotome and stained with azure B and methylene blue (1:1 v/v). The sections were examined with a Wild M-20 compound microscope.

Organoleptic Pungency. A Scoville heat unit test (Scoville, 1912) for pungency was conducted by using the four cultivars grown as Weslaco, TX. The ASTA (1968) analytical methods for pungency in Capsicum spices were used. Scoville units were calculated from the ASTA schedule B.

Statistical Evaluation. Analysis of variance was used to determine differences between cultivars in capsaicin content and lipid content. Pearsons correlation coefficients were determined for objective and subjective measurements.

RESULTS AND DISCUSSION

Capsaicin. The four cultivars of dried ground peppers used in the gas–liquid chromatographic analyses were similar to capsaicin content. When analysis of variance

Table I. Average Values for Objective and Subjective Tests of Peppers

cultivar	extractable cross wall lipids, mg/100 g	ether extracts of whole dried peppers, g/100 g	capsaicin, mg/100 g	Scoville heat units of whole dried peppers
TAM	343.60	3.614	110.0	10 000
TMJ	476.00	2.977	117.2	12 500
T85	590.00	3.602	382.0	17 000
JM	698.80	3.128	726.0	36 700
BELL	5.57			

was performed on all four cultivars, the differences were not as significant ($P > F = 0.396$). This may have been due to the extended time of heating (4–5 h) used in the extraction procedure.

Good separation of individual capsaicin components in fresh pepper extract was achieved by using gas–liquid chromatography. The fresh jalapeno extract contained greater amounts of nordihydrocapsaicin, dihydrocapsaicin, homocapsaicin, and homohydrocapsaicin in relation to the capsaicin component than the dried pepper extract. Bell pepper extract did not contain measurable amounts of capsaicin analogues.

Lipids. The total lipids of the interlocular septae of the four cultivars were measured on a fresh weight basis and significant differences were found between cultivars ($P > F = 0.0001$). The mean values are shown in Table I. Bell peppers contained less quantites of measurable lipid in the interlocular septae than jalapenos.

The ether-extractable material in whole peppers was measured on a dry weight basis. Significant differences were not found between cultivars ($P > F = 0.876$). Average values are shown in Table I. This was probably due to the high lipid content naturally found in pepper seeds (28.5%) (Lee et al., 1975).

Thin-Layer Chromatography. Lipids washed from jalapeno blisters separated into three components: capsaicin, neutral lipids and pigments, and galactolipids. Bell pepper blisters also separated into three components: neutral lipids and pigments, galactolipids, and an unidentified component that appeared as a very faint spot with the same R_f value as capsaicin.

Histology. Viewed with a binocular compound microscope, the surfaces of the cross walls of fresh hot peppers contained large blister-like protrusions amid yellow pigmented areas that appeared to be filled with a lipophilic substance. The area of blister formation extended from the axillary end to the distal end of each cross wall on both lateral surfaces. A cross section through the cross wall revealed that there was no penetration of the pigmented area or the blisters into the mesophyll layers.

Bell peppers have similar deeply pigmented areas on their cross wall surfaces. Examination of the cross walls of fresh bell peppers with a binocular compound microscope revealed blister-like protrusions; however, when ruptured, they did not appear to contain an oily substance. Instead, they were composed almost entirely of a solid mass that was sectioned easily with a razor blade. Small areas did, however, have blisters which contained an oily substance. The areas of pigmentation in bell peppers did not extend the full length of the cross walls and were confined to relatively small areas. Some cross walls of the bell peppers examined did not have pigmented areas. Very little difference was apparent upon examination of bell and jalapeno pepper cross walls with the scanning electron microscope.

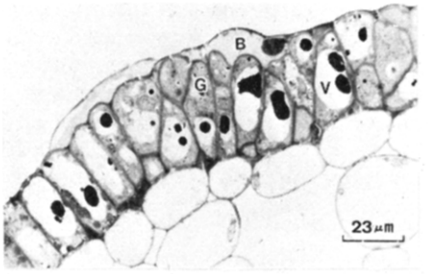

Figure 1. Light micrograph of the glandular cells of the interlocular septum of a bell pepper. Note the dense (black) osmiophilic material in the vacuoles. B = blister; B = gland cell; V = vacuole.

The glanular cells fixed with glutaraldehyde were examined with bright-field microscopy, and differences and similarities between bell peppers and jalapeno peppers became apparent. In a early secretion phase, cells in both pepper types contain a dense cytoplasm with many small vacuoles. As the pepper matures, the small vacuoles merge to form one large vacuole that fills the majority of the gland cell.

During the secretion phase, bell pepper vacuoles contain large globules of dense osmiophilic material (Figure 1). This substance is probably not lipophilic but rather a protein or tannin complex. As the secretion phase progresses, there is not a significant decrease in the quantity of this nor its globular appearance in bell peppers. Jalapeno pepper vacuoles also contain a densely osmiophilic substance; however, as the secretory phase progresses, the substance becomes scattered within the vacuoles and the total amount appears to decrease.

There is a great amount of variation in structural characteristics of the glandular regions of peppers. Jalapeno peppers generally have a monolayer of gland cells. However, bilayers of cells were found in some specimens. Two specimens (both in the ripening phase) were found to have trichome structures encased by blisters. The formations had basal stalk cells with secretory cells seated on their apexes. The secretory cells contained an osmiophilic substance. This type of formation was not found in bell peppers which had either monolayers, bilayers, or multilayers of glandular cells.

The formation of the blister appears to be the same in both pepper types. As the lipophilic substance is formed, it is secreted on several sides of the cell, but the majority is pushed out toward the apex. The cuticle is subsequently separated from the secretory cells due to pressure of the secretion and a subcuticular space is formed, the cuticle becoming a container for the secretions.

As the pepper ripens, the glandular cells progress through their secretory phase. Characteristically, the gland cells lose contact with the cells subjacent to them due to pressure created by secretory products or some other mechanism and degenerate.

There were no apparent differences between the intracellular structures in the two pepper types when viewed with transmission electron microscopy. Cells in an early secretion phase were observed by using transmission electron microscopy. They were found to contain many amoeboid-shaped plastids with a relatively dense stroma and numerous tubuli which encircle the plastids. The amount of subcuticular space formed can be considered a measure of the functional age of the gland cell (Amelunxen, 1965). Numerous mitochondria are present and many small vacuoles appear scattered throughout the cytoplasm and are not membrane bound. Secretion product collects and a space is formed between adjacent cells. Few

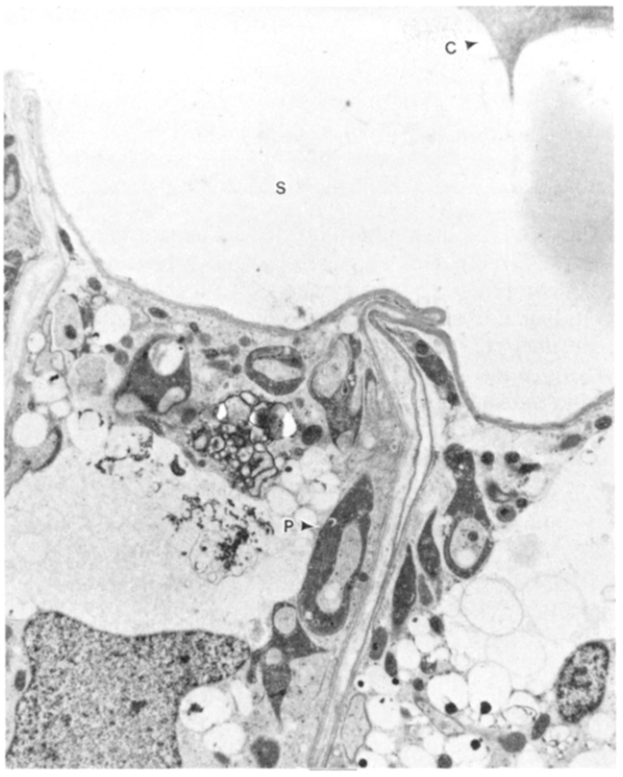

Figure 2. Transmission electron micrograph of a gland cell in a jalapeno pepper during a late secretion phase. Note the enlarged subcuticular space. P = plastid; C = cuticle; S = subcuticular space. Magnification = 3300×.

dictasomes are apparent and the endoplasmic reticulum is not prevalent.

In a later secretion phase, the subcuticular space is greatly enlarged (Figure 2). There are fewer mitochrondria and fewer plastids. Many of the smaller vacuoles merge to form a large central vacuole. Less osmiophilic material appears in the cytoplasm. The vacuole contains a very dense osmiophilic material.

The mechanism by which the secretory product leaves the cell is unknown in most merocrine cells that secrete lipophilic substances. Secretion product collects in a large droplet at the apex of the cell. The secretion appears to be separated from the cell cytoplasm by a membrane structure. This membrane is not apparent between the cell wall and the secretion. A possible mechanism for exit of the secretory product is the cell acts to wall the extraneous material off from the cell contents in a manner similar to the human body's ability to wall off foreign materials.

The $KMnO_4$-fixed material was viewed with bright-field microscopy to determine the exact location of all concentrated lipid material in pepper gland cells. Osmium fixation shows unsaturated lipid material but can also densely stain proteins and other compounds with unsaturated bonds. The components of bell and jalapeno blisters were found to be lipophilic. Lipid droplets were found throughout the cytoplasm of gland cells of both pepper types. The contents of the vacuoles of gland cells were found not to be lipophilic, as shown with $KMnO_4$ fixation.

Scoville Heat Unit Test. The mean values of the Scoville heat unit tests of the four cultivars of dried ground jalapeno peppers are shown in Table I.

Scoville heat units correlated well with capsaicin content for the four cultivars ($P > R = 0.9301$) but did not correlate as well with the extractable lipids of the cross wall ($P > R = 0.6275$). The correlation between organoleptic pungency and capsaicin content was higher than that found by Weisenfelder et al. (1977). They showed a correlation coefficient of 0.67 between organoleptic pungency and milligrams of capasaicin when they measured 12 cultivars of jalapeno pappers. The neutral lipid content of pepper seeds (28.5%) may affect the organoleptic pungency, especially in the whole ground pepper. Nelson (1919) thought that the solubility of the vanillyl acyl amides in different media may affect organoleptic pungency.

Capsaicin is synthesized in specialized gland cells of the merocrine type, on the cross walls of pungent peppers. These are modified epidermal cells and may be identified by a yellow to orange pigmentation. The greater the amount of secretion produced by these cells, the greater the amount of capsaicin and of organoleptic pungency. Other lipid substances are produced by these cells besides capsaicin and may or may not affect the organoleptic pungency.

Registry No. Capsaicin, 404-86-4.

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