Fruit Composition, Tissues, and Localization of Antioxidants and Capsaicinoids in Capsicum Peppers by Fluorescence Microscopy

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Abstract
Capsicum peppers are a fair proportion of total vegetables consumed in daily diets around the world. Whether the pepper type is non-pungent or spicy, the nutritional contribution of peppers to diet and human health is significant. However, the selectivity among seeds, carpal and placental tissues or pericarp tissues, makes a difference in the product harvest and quality of antioxidants and capsaicin. Despite documentary evidence of differences in nutritional status among pepper types, results that qualitatively compare capsaicin or antioxidant levels in different pepper fruit tissues are scarce. Analyses of microscopic images of varieties of tissues of pepper fruits were carried out with equipment at the Microscopic Imaging Laboratory at the Eastern Regional Research Center (ERRC), Wyndmoor, PA. Various other biochemical tests were carried out at the ERRC and Delaware State University to characterize capsaicinoids, antioxidants, and other biomolecules. Major differences were found among types and varieties. Work is continuing to characterize the tissue concentration and distribution of antioxidant and capsaicin contents in seeds, placental and pericarp tissues, especially in comparing non-pungent and pungent pepper types. Fluorescence images show significant differences among seeds, placenta, and pericarps. Most comparisons limit their discussion to capsaicin, but capsaicinoids and other important biomolecules exist, and their incidence needs better characterization. Fluorescence microscopy provides measures of these compounds and quantifies nutritional contents; results help in developing a more complete picture of the contribution of peppers to human health and nutrition. These characterizations using fluorescence microscopy are presented in this paper.

INTRODUCTION
Capsicum pepper fruits contribute significantly to diets around the world, and California and Florida are the leading Capsicum pepper producers in the United States. Per capita consumption of Capsicum peppers increased about 50% in the United States between 1986 and 1996 (Decoteau, 2000). Furthermore, green pepper production in the United States grew from 476,500 tons in 1990 to 893,610 tons in 2005, while green pepper production in China grew from 3,119,220 tons to 12,523,000 tons over the same period (FAO, 2007).

The nutritional value, as well as the spiciness or pungency and aroma of peppers, make them quite popular in ethnic foods in the United States, and the trend in their demand is clear.

Capsaicin (trans 8-methyl-N-vanillyl-6-nonenamide), a pungent lipophilic alkaloid, is the main molecule of interest of this presentation (Fig. 1). Capsaicin significantly accounts for the pungency in hot peppers, and Capsaicin is uniquely present in Capsicum species. Scientists and medical doctors are discovering new and ingenious ways to utilize this molecule in prophylactic treatments and cures for diseases. Antioxidants are a second focus of this report. Because these two groups of biological molecules from fruit and vegetables are significant protectors against reactive oxygen species (ROS) in plant, animal, and human metabolism, there is strong interest in understanding their biosynthesis and storage in plant tissues. Typical biochemical analysis
methods exist, but modern fluorescence techniques provide new opportunities to investigate and divulge intricacies in the mechanism of synthesis and storage of capsaicin and antioxidants in *Capsicum* species.

Hence, the major objectives of our work in investigating *Capsicum* peppers were (1) to characterize the ontogenic development and storage of desirable commercial products in the pepper fruit; (2) to search for which tissues are involved in the synthesis of such capsaicin and antioxidants; and (3) to begin the search for the mechanism of mobilization, transport, and storage of these products in the pepper fruit.

**MATERIALS AND METHODS**

Our work focused on producing and securing healthy pepper fruits of *Capsicum annuum* var. Bell pepper fruits and *Capsicum chinense* var. Habanero. These fruits were studied fresh and after refrigeration. Some fruits were frozen for later examination, and others were stored in glutaraldehyde for future analyses. Microscopy imaging was central to our investigations.

Work began with visual inspection using a compound microscope, and fruits and their tissues were selected for further analyses. Some tissues were further processed by staining and examining with various light filters. A dipfluorescence microscopy was used to determine the localization of fluorescent compounds. According to Rost (1992) and Rost (1995), only certain molecular species provide the requisite emissions we sought. Capsaicin is one of those distinctive molecules. The confocal laser scanning microscope provided clear color images of the localization of molecular deposits, and the transmission electron microscope (TEM) and the scanning electron microscope (SEM) added sub-cellular details.

**RESULTS AND DISCUSSION**

Despite the size difference between the Bell and Habanero peppers, the similarity in the architecture of the two peppers was clear. The first discovery was the difference in the support structure of the walls of the large Bell pepper in contrast to the wall of the smaller Habanero pepper. Figure 2 provides fluorescence images of the two structures. In the image of the Bell pepper, angular beam structures hold up the wall of the pepper, but no such image is seen in the smaller sized Habanero pepper wall structure.

Our results showed blistered structures, which we identified as glands, or vesicles in placental tissues in Habanero fruits. Only trace structures were seen in placental tissues or carpal tissues in Bell fruits (Fig. 3). The results are similar to others who found that “blistered structures” were only present in the pungent type genotype, but they were entirely absent from the non-pungent type (Stewart et al., 2007). Our results go a little further in beginning to answer the question posed by Stewart et al. (2007) when they wrote that “…it is not known whether capsaicinoids are transported intercellularly from nearby cells not immediately under a blister”. The Syto 9 and Nile Red stained placental cells in our study clearly show the nuclei (stained green) and the lipid capsaicinoids (stained red) which mobilized out of the epidermal cells of placenta tissues and deposited outside of the cell walls, where they are apparently stored in the apoplast - out of the active symplasm (Fig. 4). Figure 5 shows the electron micrographs that examine cells and tissues for the stored compounds. More studies of the phenomenon are being pursued, and Figure 6 is a photograph that compares the size of the smaller Habanero pepper fruits with the large Bell pepper fruit.

Because capsaicinoids are produced by condensation of a branched fatty acid (8-methyl-6-nonenoyl-CoA) with vanillyamine, there is obviously some competition for reactants to the production of typical antioxidants versus the alkaloid capsaicin, and a wide gamut of scientists agree that very little is known about the regulation of the biosynthesis of capsaicinoids, especially in comparison to the production of antioxidants. Figure 7 is a graph, developed from data acquired by Decoteau (2000). It shows a comparison of the level of antioxidants in pungent versus non-pungent *Capsicum* peppers, but many reports confirm the richness of antioxidants in *Capsicum* peppers in comparison
to citrus and many other plant species. Howard et al. (2000), nevertheless, reported that more research is needed to understand the complex interactions that occur among the various antioxidants present in pepper extracts. More work is needed, especially with fluorescence imaging of antioxidants.

New knowledge about antioxidants and capsaicin in peppers opens new questions about potential uses of other compounds in the Solanaceae, compounds that include atropine, nicotine, and a variety of other biologically active molecules. Antioxidants are recognizably a major contribution from peppers, but their production is only an introduction of what potential lies in the Nightshade family. With the current prominence of China as a major Capsicum pepper consumer, it is time to learn more about how the demand for peppers in the United States, China, and around the world can be met.

Capsaicin, on the other hand, is a food, a spice, a food flavoring agent, and a pain killer. As a pain-killer, it is the active ingredient in many muscle pain relief medications, including the commercial product “Icy Hot.” In medicine, many recent reports discuss the use of capsaicin to create a feeling that is ‘filling,’ establishing a ‘satiation’ factor. Several reports present capsaicin as a promising compound in research to control obesity (Mela, 2005; Diepens et al., 2007). Other recent reports are touting results that capsaicin is an effective regimen in killing prostate cancer cells and controlling prostate cancer disease (Mori et al., 2006).

In conclusion, our work may impact the produce industry. Understanding the mobilization and storage process of capsaicin pepper fruit tissues would stimulate changes from crushing whole fruits to securing placenta tissues for effective harvesting and quality processing of pepper fruits for capsaicin. Harvest dates would be determined more precisely per production and motility cycling for antioxidants and capsaicin in pepper fruits.

There is also an impact on consumer health. In terms of healthfulness, the maxim “prevention is better than cure” is a statement of significance. The increasing consumption trend for peppers provides a continuous supply of antioxidants whose various benefits are not all yet quantified. The medicinal benefits of capsaicin are only now being elucidated, and new reports are quite encouraging.

**Literature Cited**


Figures

Fig. 1. The molecule of capsaicin (trans 8-methyl-N-vanillyl-6-nonenamide).

(a)       (b)

Fig. 2. Fluorescence images of the structures of the Bell pepper (a) with angular beams that hold up the wall of the pepper; no such beam structure is seen in the walls of the smaller sized Habanero pepper fruit (b).

(a)         (b)

Fig. 3. Glands or vesicles are clearly visible by fluorescence imaging in placental tissues in Habanero fruits (a). Only trace or vestigial vesicle structures were seen in placental tissues or carpal tissues in Bell fruits (b).
Fig. 4. Nile Red stain clearly shows where lipophilic capsaicinoids are mobilized out of the cells and deposited outside the cell walls, where they are apparently stored in the apoplast - out of the active symplasm. The magnified image to the right shows details of the stained tissues.

Fig. 5. Electron micrographs of cells and tissues show stored capsaicinoid compounds. More studies of the phenomenon are being pursued. The right image shows a close-up of the active state of epidermal cells in the placenta.

Fig. 6. This photograph compares the size of the smaller Habanero pepper fruits with the large Bell pepper fruit.
Fig. 7. A comparison of Vitamin C (antioxidants) in pungent versus non-pungent Capsicum peppers. Our results were more qualitative, but those of Decoteau are a good comparison. The difference in the level of capsaicin synthesized may definitely have an effect the quantity of antioxidant produced in the pepper fruit.