Capsaicinoids in Chili Pepper (*Capsicum annuum* L.) Powder as Affected by Heating and Storage Methods

Y. Wang, Y. Xia, J. Wang, F. Luo, Y. Huang

Abstract. *Loss of capsaicin and dihydrocapsaicin is a problem in the preparation and storage of dehydrated peppers. In this study, the effects of heating (100°C to 260°C, 15 min) and storage conditions (vacuum vs. non-vacuum package, room temperature vs. refrigeration temperature) on the content of capsaicin and dihydrocapsaicin in chili pepper powder were studied with pungent components assayed by HPLC. Heating between 100°C and 190°C for 15 min did not seriously affect the content of capsaicinoids (<10% reduction). However, at temperatures greater than 190°C, capsaicin and dihydrocapsaicin degraded rapidly. Low-temperature storage improved retention of capsaicinoids, and vacuum packaging provided little if any extra benefit. Capsaicin and dihydrocapsaicin had very similar stability and showed similar reduction rates during heating and storage, which indicates that the C=C bond in capsaicin is relatively stable and does not account for its decomposition during heating and storage.*

Keywords. *Capsaicin, Capsaicinoids, Dihydrocapsaicin, Storage, Temperature, Vacuum package.*

Chili peppers are important food widely consumed around the world. They are consumed directly as vegetables or processed as spices in dried and powder forms. Chili is also a primary ingredient in various hot sauces and pastes in different cuisines. The unique pungent flavor of chili peppers is due to capsaicinoids, a family of compounds consisting of acid amides of vanillylamine and a C_8-C_{13} branched-chain fatty acid (Kobata et al., 1999) (fig. 1). Capsaicin and dihydrocapsaicin are two primary capsaicinoids, accounting for about 90% of the total capsaicinoids in chili peppers (Korel et al., 2002). Small amounts of other capsaicinoids, such as nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin, are also present in chili peppers and account for less than 10% of the total capsaicinoids. Since the pungency strength is a critical quality parameter for chili pepper products, the stability of capsaicinoids is one of the major concerns with fresh chili peppers and with their processed products.

Various factors affect the stability of capsaicinoids and result in a decrease of capsaicinoids in chili peppers or products containing chili pepper. While the amount and profile of capsaicinoids are related to genetics (e.g., species and varieties) and physiology (e.g., stages of maturity), they are also affected by storage environment and processing methods (Contreras-Padilla and Yahia, 1998; Kirschbaum-Titze et al., 2002a). In fresh chili pepper, oxidation catalyzed by peroxidases is one of the major factors causing a decrease in capsaicinoid levels (Kirschbaum-Titze et al., 2002b; Goodwin and Hertwig, 2003). Capsaicinoids in minced chili pepper are normally more susceptible to oxidation than those in intact chili pepper, since the disruption of plant tissues allows an enhanced contact between peroxidases and capsaicinoids. Therefore, applying blanching to inactivate peroxidases or storing the product in a nitrogen atmosphere can help to retain capsaicinoids, although some peroxidase activity may occur after a relatively severe temperature process (Schweiggert et al., 2006). Other processing methods, such as gamma irradiation, may also help to retain capsaicinoids in chili pepper (Topuz and Ozdemir, 2004).

It is well known that post-harvest applications affect the content of capsaicinoids in chili peppers and their products, yet there are very limited studies on how capsaicinoids are affected by specific thermal processing techniques or storage methods, particularly for roasted dried chili pepper and spice

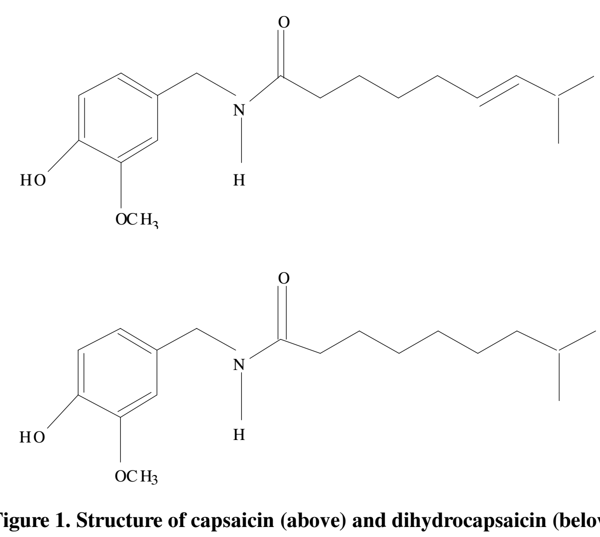

Figure 1. Structure of capsaicin (above) and dihydrocapsaicin (below).

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powder. The objective of this study was to determine the effects of processing temperature and storage conditions on the content of capsaicin and dihydrocapsaicin, two primary capsaicinoids in chili pepper powder. Understanding how these processing parameters could be optimized to retain capsaicinoids is essential to ensure the quality of chili pepper powder and similar food items as well as the quality of fried and baked foods containing these ingredients.

Materials and Methods

Sample Preparation

During our previous studies, we tested 12 different types of chili pepper from different suppliers. Among them, a wild chili pepper species had very high levels of capsaicin (19.20 g kg^{-1}) and dihydrocapsaicin (6.35 g kg^{-1}), but it was rare and difficult to acquire. Most of the other chili peppers had relatively low amount of dihydrocapsaicin (0.01 to 1.27 g kg^{-1}), which may be undetectable after heating at some selected test temperature for a short time. We chose a rod chili pepper, Chao-tian-jiao (*Capsicum annuum* L. var. *conoides*) provided by Changsha La-Mei-Zhi, Ltd. (Hunan, China) for our experiments. The rod chili pepper is widely cultivated in south China, such as in Hunan, Yunnan, and Fujian provinces. The rod chili pepper is about 2.5 to 5.0 cm long, 2 to 5 g, and contains relatively large amounts of capsaicin (4.25 g kg^{-1}) and dihydrocapsaicin (2.51 g kg^{-1}). It is often used as an additive for standardizing the spiciness of hot sauces and hot pastes in China.

Chili Pepper Powder Preparation

Fresh chili pepper was oven-dried at 50°C until the water content was reduced to 12% to 13%, which took about 6 to 8 h. The dried pepper was then ground (FW177, Tianjin Taisite Instrument Co., Ltd., Tianjin, China) until the powder could pass through a sieve (Hebei An-Ping-Sheng Special Screen Materials, Heibei, China) with a nominal mesh aperture of no more than 0.391 mm. The powder was stored at refrigeration temperature for later experiments.

Effect of Heating Temperature

Chili pepper powder (2.500 g) was placed into a 100 mL beaker and heated in an oven (SL, Shanghai Laboratory Instrument Works Co., Ltd., Shanghai, China) at a selected temperature (100°C to 260°C at intervals of 10°C) for 15 min, and then analyzed for its capsaicin and dihydrocapsaicin content.

Effect of Storage

Chili pepper powder samples were vacuum packed in polyethylene bags (500/S, Shanghan Ruida Packaging Food Machinery Co., Ltd., Shanghan, China; residual gas pressure 0.09 mPa) or non-vacuum packed in polyvinyl chloride bags, and then stored at room temperature (5°C to 35°C) or refrigeration temperature (about 2°C to 5°C) for nine months. The room temperature (5°C to 35°C) was the indoor temperature during our nine months of study. In most of south China, indoor temperatures are normally uncontrolled due to lack of heating and air conditioning; therefore, using an uncontrolled room temperature (5°C to 35°C) in our experiments helped us to better understand the changes in capsaicinoids in dried pepper that occur at common storage temperatures in our region. Samples were covered with a black plastic bag to avoid exposure to light. The content of capsaicin and dihydrocapsaicin in chili pepper powder was determined every month by high-performance liquid chromatography (HPLC).

HPLC Analyses

A modified ISO method (ISO, 1993) was used to determine capsaicin and dihydrocapsaicin contents, in which ultrasonication instead of Soxhlet extraction was used to extract capsaicinoids from the chilis. Soxhlet extraction required 8 h, while ultrasonication took about 1 h. Using ultrasonication can significantly reduce the extraction time (Wang et al., 2006).

Extraction

Chili pepper powder (2.500 g) was mixed with 25 mL of methanol-tetrahydrofuran solvent (1:1 v/v) in a 100 mL beaker. The mixture was sonicated (KQ2200DE, Jiangsu Kunshan Ultrasonic Instruments, Ltd., Kunshan, China) for 30 min at 60°C, filtered through filter paper, and the filtrate was collected. The residue was re-extracted two more times with 25 mL of solvent and 10 min of sonication, and the filtrates were pooled (approx. 75 mL). The filtrate was concentrated at 70°C in a water bath, transferred to a 50 mL volumetric flask, and made up to the mark with methanol-tetrahydrofuran. This filtrate was filtered through a 0.45 μm membrane in preparation for HPLC analysis.

Determination of Capsaicinoids

An Agilent 1100 HPLC (Agilent, Waldronn, Germany) with UV detector and an Agilent Zorbax SB-C18 column (5 μm, 250 × 4.6 mm) were used for HPLC analysis. The injection volume for capsaicinoid extract was 10 μL, and the column temperature was 30°C. The mobile phase was methanol and water (65:35 v/v), and the flow rate was 1.0 mL min^{-1}. The detection wavelength was 280 nm. The contents (W, mg g^{-1}) of capsaicin and dihydrocapsaicin in chili pepper powder were calculated as:

$$W = \frac{C \cdot V}{m} \tag{1}$$

where C is the concentration (mg mL^{-1}) of capsaicin or dihydrocapsaicin calculated from the calibration curves for standard capsaicin and dihydrocapsaicin, V is the total volume of extract (50 mL in this study), and m is the sample weight (2.500 g in this study). Replicate samples were prepared for each treatment (N = 2) with capsaicinoid content determined in triplicate for each sample.

Statistical Analysis

Analysis of variance (SPSS 12.0, SPSS, Inc., Chicago, Ill.) was conducted to determine whether storage temperature and package caused significant difference ($\alpha = 0.001$) in capsaicinoid content (sum of capsaicin and dihydrocapsaicin) during nine months of storage.

Results and Discussion

Figure 2 provides representative HPLC chromatograms for standard capsaicin and dihydrocapsaicin as well as for capsaicin and dihydrocapsaicin extracted from chili pepper

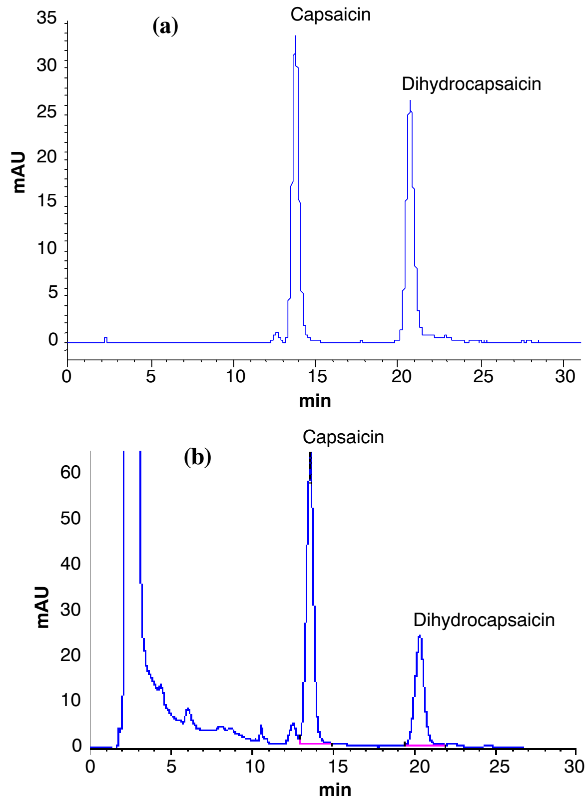

Figure 2. HPLC chromatograms for capsaicin and dihydrocapsaicin: (a) standard, and (b) extracted from chili pepper powder. The limit of detection was 100 ng mL^{-1} for both capsaicin and dihydrocapsaicin.

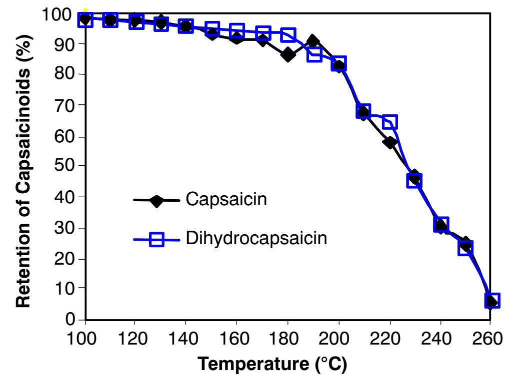

Figure 3. Retention of capsaicin and dihydrocapsaicin in chili pepper powder after heating at 100°C to 260°C for 15 min.

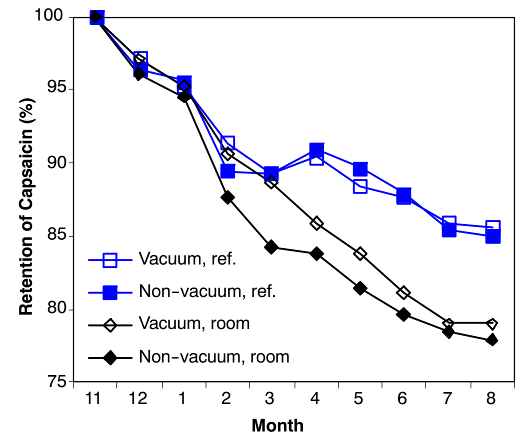

Figure 4. Retention of capsaicin during nine months of storage (ref = 2°C to 5°C, room = 5°C to 35°C).

powder. The contents of capsaicin and dihydrocapsaicin in chili pepper powder used in this study were 0.396% to 0.435% and 0.192% to 0.261% on a dry weight basis, respectively, with variation dependent primarily on biological variation between lots of chili peppers and storage time. The relative standard deviation ($n = 10$) was 2.74% for capsaicin determination and 2.38% for dihydrocapsaicin.

Effects of Heating

After heating for 15 min at 100°C, the amounts of both capsaicin and dihydrocapsaicin in dried chili pepper decreased less than 2%, quite different from the study results reported by Schweiggert et al. (2006). Schweiggert et al. (2006) heated whole or minced fresh chili pepper at 80°C to 100°C for 5 to 10 min and observed a 20.4% to 30.2% reduction in capsaicin and a 25.6% to 34.6% reduction in dihydrocapsaicin content. This suggests that the process of an initial dehydration step followed by drying at higher temperature may result in a more stable dried chili powder. Treatment of the fresh chili pepper at a high temperature may have led to a greater loss due to the enzymatic or chemical oxidation of heat-sensitive capsaicinoids.

Heating partially dehydrated chili at temperatures between 100°C and 190°C did not result in significant loss of capsaicinoids, with a reduction at or below 10% over this wide temperature range. However, heating at temperatures greater than 190°C resulted in rapid decomposition of capsaicin and dihydrocapsaicin (fig. 3). For example, the amounts of capsaicin and dihydrocapsaicin decreased 17.50% and 16.05%, respectively, after heating at 200°C, decreased 53.18% and 54.91% after heating at 230°C, and decreased 94.32% and 93.19% after heating at 260°C. The boiling points of capsaicin and dihydrocapsaicin are 210°C to 220°C (Di et al., 1999), so evaporative losses above a heating temperature of 210°C would be expected.

To make deep-fried foods, baked products, or roasted meats, a temperature at or above 200°C may be required. If chili pepper is used in these products, then the decomposition of spicy compounds should be compensated for if foods with a consistent flavor are to be produced.

Effect of Storage

Figures 4 and 5 show the contents of capsaicin and dihydrocapsaicin in chili pepper powder at different storage times and under different packaging atmospheres. The amounts of capsaicin and dihydrocapsaicin decreased continuously during nine months of storage, and dihydrocapsaicin showed a somewhat higher reduction rate than capsaicin (figs. 4 and 5). Dihydrocapsaicin decreased 20.6% to 21.2% at refrigeration temperature (2°C to 5°C) and 28.8% to 29.9% at room temperature (5°C to 35°C), while capsaicin decreased 14.5% to 15.0% at refrigeration temperature and 20.9% to 21.9% at room temperature. In addition, during the first three months of storage, the amount of capsaicinoids decreased much faster than in the later stage. The levels of capsaicin and dihydrocapsaicin decreased 8.6% to 12.2% and 12.5% to 13.3%, respectively, in three months. The rapid decrease of the capsaicinoid content in chili pepper powder during the first three months of storage may have been due to residual enzymatic-

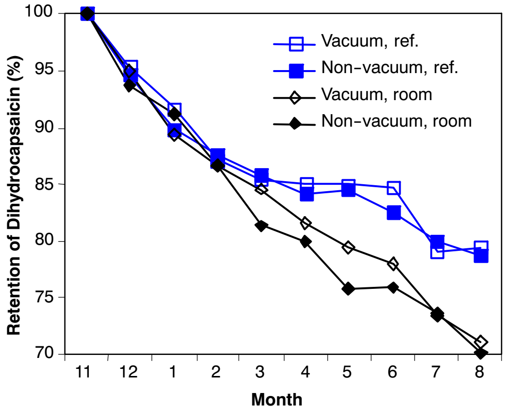

Figure 5. Retention of dihydrocapsaicin during nine months of storage (ref = 2°C to 5°C, room = 5°C to 35°C).

induced oxidation. Some heat-resistant enzymes may be released from damaged cells due to grinding (Kirschbarum-Titze et al., 2002b) and remain active after oven-drying, resulting in greater losses during the early storage period.

The result of ANOVA showed that storage temperature led to significant difference in capsaicinoid content in chili pepper. A low-temperature storage condition helped to retain capsaicinoids (figs. 4 and 5). During the first three months of storage (from November to February, no heating), the room temperature (approx. 5°C to 10°C) was similar to the refrigeration temperature. Therefore, changes in the levels of capsaicinoids (sum of capsaicin and dihydrocapsaicin) were similar between two temperature treatments during the first three months. However, with an increase in room temperature from February onward due to weather change, the chili pepper powder stored at refrigeration temperature retained a higher level of capsaicinoids. The level of capsaicinoids in chili pepper powder stored at refrigeration temperature decreased 16% to 17% after nine months, compared to a 23% to 24% reduction for that stored at room temperature. Low temperature inhibited or slowed enzymatic and/or non-enzymatic reactions, as would be expected.

There was no significant difference in capsaicinoid content between vacuum and non-vacuum packaged samples during the nine-month storage period, and only about a 1% difference was observed between vacuum and non-vacuum treatments. This result was unexpected and seemed to contradict the results from the study by Kirschbaum-Titze et al. (2002b), who found that fresh minced chili pepper stored in nitrogen retained a much higher level of capsaicinoids than that stored in atmospheric conditions. This difference may be a reflection of the moisture content of the samples in the two studies. Dried chili pepper powder may have much less active peroxidase than fresh chili.

Conclusion

Thermal decomposition of capsaicinoids in dehydrated chili was observed, particularly when temperatures reached 190°C or above. Storage temperature affected the stability of capsaicinoids, with samples stored at lower temperatures having greater capsaicinoid retention. Packaging in air or vacuum did not appear to affect storage stability. Unsaturated C=C bonds in capsaicin did not adversely affect the stability of capsaicinoids. Although capsaicin (with C=C) and dihydrocapsaicin (without C=C) had similar stability and showed similar reduction rates during a 15 min heating process (100°C to 260°C), capsaicin showed a somewhat higher retention rate than dihydrocapsaicin during a nine-month storage period. Similar results were observed by Topuz and Ozdemir (2004). Further study needs to be conducted to understand the mechanisms of the decomposition of capsaicinoids during processing and storage.

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