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Variability in capsaicinoid content and Scoville heat ratings of commercially grown Jalapeño, Habanero and Bhut Jolokia peppers

Ken G. Sweat, Jennifer Broatch, Connie Borror, Kyle Hagan, Thomas M. Cahill *

School of Mathematical and Natural Sciences, Arizona State University, West Campus, 4701 W Thunderbird Rd, Glendale, AZ 85306, United States

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The variability of capsaicinoid content of three common, commercially-available hot pepper varieties, namely Jalapeño, Habanero and Bhut Jolokia, was investigated. For each variety tested, ten peppers were acquired from each of ten different suppliers resulting in 100 peppers per variety that were individually analyzed. The results showed that different pepper varieties had different distribution types. The Habanero peppers showed a normal distribution; the Bhut Jolokia showed a skewed distribution and the Jalapeño peppers showed a very skewed distribution. The source of variability was also different; the Habaneros were very consistent within a given pepper supplier so most of the overall variation resulted from differences between suppliers. The Jalapeño peppers were the exact opposite with a very high degree of variability within a given supplier and relatively low variation between suppliers. A bootstrap statistical simulation was conducted on the data to suggest a minimum number of peppers to analyze to characterize the variation in a population. The simulations indicated that small sample sizes are effective at estimating the mean concentrations, but a sample size of ten or more is necessary to describe the population and capture the high-end tail of the distributions, which are the very hottest peppers.

1. Introduction

Chili peppers, the fruits of *Capsicum spp*. are used as seasonings in a wide diversity of cuisines from the Old and New World. Certain species and varieties are prized for their intense 'heat', which is imparted by capsaicin, dihydrocapsaicin and related compounds (capsaicinoids) synthesized in the fruits. Capsaicinoids are produced by a dehydration synthesis reaction involving vanillylamine and short chain fatty acids. The different capsaicinoids present are the result of the different fatty acids available for the dehydration synthesis reaction (Aza-Gonzalez, Nunez-Palenius, & Ochoa-Alejo, 2011). The capsaicinoids also have a variety of pharmaceutical applications (Meghvansi et al., 2010; Reinbach, Smeets, Martinussen, Moller, & Westerterp-Plantenga, 2009) that have resulted in interest in peppers as a source of these compounds.

Peppers have been shown to be variable with respect to capsaicin concentrations, which includes peppers harvested from the same plant (Keyhaninejad, Curry, Romero, & O'Connell, 2014; Kirschbaum-Titze, Heipler, Mueller-Seitz, & Petz, 2002). This has spawned a series of research projects that tested various factors that might increase capsaicinoid production. These factors include: osmotic stress (Arrowsmith, Egan, Meekins, Powers, & Metcalfe, 2012; Kehie, Kumaria, & Tandon, 2012), harvest season (Pordesimo, Li, Lee, & Reddick, 2004), nitrogen fertilization (Medina-Lara, Echevarria-Machado, Pacheco-Arjona, Ruiz-Lau, & Guzman-Antonio, 2008; Monforte-Gonzalez, Guzman-Antonio, Uuh-Chim, & Vazquez-Flota, 2010), node number (Mueller-Seitz, Hiepler, & Petz, 2008; Zewdie & Bosland, 2000a, 2000b), age of fruit (Contreras-Padilla & Yahia, 1998; Iwai, Suzuki, & Fujiwake, 1979; Mueller-Seitz et al., 2008). Given the large number of parameters that may affect capsaicinoid production, it is expected that cultivation conditions may affect capsaicin production. Multiple studies have assessed the degree of variation introduced by environmental factors and environmental-genomic factor interactions (Butcher et al., 2012; Gurung, Techawongstien, Suriharn, & Techawongstien, 2012; Harvell & Bosland, 1997; Zewdie & Bosland, 2000a, 2000b).

* Corresponding author.
E-mail address: tmcahill@asu.edu (T.M. Cahill).

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While most of the previously cited research has been done on accessioned varieties grown under experimentally controlled conditions, a handful of studies have analyzed the capsaicinoid content of fruits purchased in commercial markets. These "market basket" experiments are valuable since they describe the actual fruit that the public consumes. Total capsaicin content of fruit was determined for twelve local varieties of chilies purchased in Caringin Central Market, Bandung, West Java (Indonesia) (Musfiroh, Mutakin, Angelina, & Muchtaridid, 2013). Analysis of capsaicin and dihydrocapsaicin content of the fruit pericarp, placenta and seeds for multiple pepper varieties was determined for peppers purchased in Yucatan (Cisneros-Pineda et al., 2007) and Chihuahua, Mexico (Orellana-Escobedo, Garcia-Amezquite, Olivas, Ornelas-Paz, & Sepulveda, 2012). However, none of these studies directly assessed variability in detail within a particular strain of chili peppers.

Despite the plethora of research articles on capsaicinoid concentrations, the fundamental distribution and variation in capsaicinoid concentrations in even some of the common pepper varieties is surprisingly vague. This is largely due to the analytical procedures utilized by different research groups. Many researchers conduct only triplicate analyses on each pepper type or condition, which is enough to estimate the mean concentration of the population but not to determine the variability in the population. Other research groups combine many peppers into a single sample, which is then homogenized. Effectively, this is a physical averaging of the individual peppers that is an efficient means to determine the average concentration of a population of peppers, but it completely obscures information about the variability in the population. In some articles, three replicate analyses were conducted using the same sample homogenate so the resulting standard deviation reported is representative of the analytical variability rather than variability of the pepper population. To the best of our knowledge, no population distributions, as illustrated by histograms, of pepper heat have been reported in the literature.

The objective of this research is to determine the fundamental distribution of capsaicinoids, and hence Scoville Heat Units (SHU), in three commonly available commercial pepper varieties, namely the Jalapeño, Habanero and Bhut Jolokia. To estimate the representative cross-section of peppers available to consumers, ten peppers from each of ten different suppliers were purchased and analyzed, so 100 peppers were individually analyzed for each variety. Statistical tests, such as ANOVA, were performed to determine the amount of variability that could be attributed to the peppers themselves and the degree of variability imparted by different growing conditions or pepper strains used by the different suppliers. Lastly, bootstrap simulations were conducted with the sampled population to gain insight into the minimum sample size to determine the average heat level in a population as well as to estimate the minimum sample size necessary to assess the maximum range of heat possible for the population. Non-parametric methods such as the bootstrap are appropriate for measuring statistics (e.g. maximum) for which the distribution is not defined.

2. Materials and methods

Three varieties of commonly available hot peppers were selected for this experiment, namely the Jalapeño, Habanero and Bhut Jolokia. The Jalapeño and Habanero have a large body of literature data in terms of their capsaicin and dihydrocapsaicin concentrations, so an extensive metadata analysis can be conducted to complement the variability study. The Bhut Jolokia has only recently received attention since it is among the hottest pepper varieties (Bosland & Baral, 2007), so data on this variety are more limited.

2.1. Sample size and preparation

One hundred peppers from each variety were purchased and analyzed to estimate the variability of SHUs in the population. To estimate the differences between different growers, ten peppers were obtained from each of ten different sources. For the Jalapeño peppers, all the peppers were purchased as fresh peppers from ten different stores in the Phoenix metropolitan area of Arizona. While it is possible that some of the stores used the same pepper suppliers, it is unlikely that most of the stores had the same pepper source, therefore the sample should represent a reasonable cross section of the commercially available peppers. Personal communication between the authors and the owner of a local farm indicated it was unlikely that the different stores used the same source. The Habanero peppers were likewise locally purchased, but two of the ten suppliers sold the peppers as pre-dried fruit rather than fresh fruit. The Bhut Jolokia peppers were a specialty item and were not available at enough local stores to achieve the desired sample size. Therefore, dried pepper pods were purchased through the internet from ten different suppliers. The internet suppliers ranged from large, international specialty produce corporations to small hobbyist growers. Most suppliers were small commercial scale producers. Peppers had been purchased from an additional two suppliers, but the peppers received did not match the size, shape or aroma of the Bhut Jolokia peppers and hence they were considered miss-labeled and not analyzed.

Each pepper pod was analyzed separately to determine the variation in the population. The fresh peppers were cut lengthwise into quarters and allowed to air dry at room temperature (~22 °C) until they appeared dry. Previous analysis demonstrated drying technique had minimal, if any, effect on capsaicinoid content (see Supporting Information Section S-3). The peppers that were purchased as dried fruit were processed as received. The dried peppers were then weighed to determine their initial mass. Each pepper was separately ground into a powder. A 0.1 g aliquot of the powder was removed for extraction and analysis. The remaining powder was weighed, placed in a glass vial, and dried for 24 h at 85 °C to obtain the formal wet weight/dry weight ratio for each sample. The average moisture content was 4.2 ± 1.9% for the Habaneros, 4.8 ± 2.0% for the Jalapeños and 6.5 ± 1.8% for the Bhut Jolokias. The low moisture contents were expected, since the peppers were effectively dry to begin with. All the capsaicinoid concentration data was converted to "dry weight" basis for consistency. The consistency of the analytical method was tested by analyzing ten separate aliquots from a single, ground Habanero pepper sample. These ten replicate measurements determined the degree of variability introduced in the data set by the analytical method.

The standard operating procedure for the pepper extraction and analysis is presented in the Supporting Information. Briefly, 0.1 g of pepper powder was added to a clean centrifuge tube along with 5 mL of toluene. The samples were shaken for one hour and then centrifuged at 1500*g* (3000 rpm) for six minutes. The supernatant was transferred to clean test tubes and then the sample solids were extracted two more times resulting in a total of 15 mL of sample extract. The extract samples were either directly added to the GC vial (Jalapeño and Habanero) or diluted with toluene to a 1/10 solution in the GC vial (Bhut Jolokia) based on expected concentration of capsaicinoids. The final volume in the GC vial was 100 μL. BSTFA (25 μL) was added to the samples and standards to derivatize the capsaicinoids to a more volatile form for GC–MS analysis (Todd, Bensinger, & Biftu, 1977). Tetracosane-d_{50} was added as an injection standard to normalize for instrument drift. The GC vials were heated at 40 °C for 24 h and then immediately placed on the gas chromatograph–mass spectrometer for analysis.