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The effect of ripening, heat processing and frozen storage on the *in vitro* bioaccessibility of capsaicin and dihydrocapsaicin from Jalapeño peppers in absence and presence of two dietary fat types

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ABSTRACT

To date, there is no information in the literature regarding the bioaccessibility of capsaicinoids from natural sources. The effect of ripening and heat-processing on the *in vitro* bioaccessibility of capsaicin and dihydrocapsaicin was studied in the absence and presence of two dietary fat types. The capsaicinoid bioaccessibility was also studied during the frozen storage of peppers for 6 months. Fresh green peppers showed the highest capsaicinoid bioaccessibility, as compared with that of other experimental groups. The bioaccessibility of capsaicinoids from green peppers decreased as the intensity of heat treatment increased. The dietary fat increased the bioaccessibility of capsaicin and dihydrocapsaicin in digestions with red peppers, especially that of dihydrocapsaicin. The bioaccessibility of capsaicinoids was altered by frozen storage. The Caco-2 cells incorporated capsaicin and dihydrocapsaicin (8.4% and 10.9%, respectively) but they were probably metabolized by cells.

1. Introduction

Interest in the pungent components of hot peppers has increased in recent years due to their benefits on human health, which include hypolipidaemic, hypoglycaemic, antioxidant, anti-inflammatory, analgesic and other properties (Bley, Boorman, Mohammad, McKenzie, & Babbar, 2012; Chaiyasit, Khovidhunkit, & Wittayalertpanya, 2009; Srinivasan, 2005). Research concerning capsaicinoids has been almost exclusively focused on the understanding of the mechanisms involved in their protective effects. Information regarding capsaicinoid bioaccessibility and bioavailability is scarce. There are a few *in vitro*, *in situ* and *in vivo* studies with rats where the bioaccessibility and bioavailability of purified capsaicin (CAP) and dihydrocapsaicin (DHC) were tested (Donnerer, Amann, Schluligoi, & Lembeck, 1990; Kawada, Suzuki, Takahashi, & Iwai, 1984; Monsereenusorn, 1980; Suresh & Srinivasan, 2007, 2010). To date, capsaicinoid bioavailability has only been studied once in humans using *Capsicum* capsules (Chaiyasit et al., 2009). The bioaccessibility and bioavailability of capsaicinoids from natural sources has not been studied, however, despite the fact that these compounds possess high importance, since the consumption of purified capsaicinoids has been associated with adverse effects on human health (Bley et al., 2012; Kawada et al., 1984). *In vitro* digestions of foods coupled to Caco-2 cell cultures have been widely used for the estimation of the bioaccessibility of different compounds, but not for capsaicinoids. These models are not expensive and allow the evaluation of the effect of individual or multiple factors involved in the digestion and absorption of dietary phytochemicals (Ornelas-Paz, Failla, Yahia, & Gardea-Bejar, 2008; Tydeman et al., 2010; Victoria-Campos et al., 2013).

The absorption of dietary capsaicinoids should be more complex than that of purified compounds. Some studies have demonstrated that the bioavailability of pure capsaicinoids is dose-dependent (Donnerer et al., 1990; Monsereenusorn, 1980). However, dose size of dietary capsaicinoids vary since the capsaicinoid content in peppers depends on many factors, including genotype, ripening stage, processing style and preservation technology. The total capsaicinoid content varies greatly between

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pepper genotypes, ranging in some cases from 1.2 to 6580 μg/g of fresh pepper (Choi et al., 2006; Pino et al., 2007). Ripening also alters the capsaicinoid content in peppers. These compounds are typically accumulated during pepper ripening, reaching a maximum concentration 40–50 days after fruit set, and then they are degraded during fruit senescence (Contreras-Padilla & Yahia, 1998; Estrada, Bernal, Díaz, Pomar, & Merino, 2000). Heat processing (boiling, grilling, drying, pressure cooking and pasteurization) and freezing may either diminish up to 50% or increase up to 26 times the initial capsaicinoid content of peppers (Daood, Kapitány, Biacs, & Albrecht, 2006; Harrison & Harris, 1985; Huffman, Schadle, Villalon, & Burns, 1978; Orak & Demirici, 2005; Ornelas-Paz et al., 2010; Schweiggert, Schieber, & Carle, 2006; Suresh, Manjunatha, & Srinivasan, 2007; Topuz & Ozdemir, 2004). Besides alteration in capsaicinoid content, heat processing, freezing and ripening modify the matrix of peppers, possibly altering the digestibility of the fruit, the release of capsaicinoids and, therefore, the bioaccessibility and bioavailability of these compounds (Harrison & Harris, 1985; Huffman et al., 1978; Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010; Schweiggert et al., 2006; Tydeman et al., 2010). These effects are mediated by the intensity and duration of treatments, the characteristics of cell-wall polysaccharides, and the intracellular linkage of capsaicinoids with other macro- and micro-molecules (Castro et al., 2008; Daood et al., 2006; Lemmens et al., 2010; Pérez-Alemán et al., 2005). Boiling and grilling are two heat-processing styles commonly used to cook Jalapeño peppers (Ornelas-Paz et al., 2010). Freezing is a popular technology commercially used to preserve the natural organoleptic attributes of Jalapeño peppers (Azcarate & Barringer, 2010). Other common preservation technologies for this vegetable are drying, dehydration, pickling and canning (Alvarez-Parrilla, de la Rosa, Amarowicz, & Shahidi, 2011; Harrison & Harris, 1985). All of these methods can also modify the matrix of the peppers and alter differentially the bioaccessibility and bioavailability of their phytochemicals. On the other hand, the co-consumption of capsaicinoids and fat might add complexity to the capsaicinoid absorption process, since these compounds are of a lipophilic nature but can be absorbed in the presence or absence of fat (Donnerer et al., 1990; Kawada et al., 1984; Monsereenusorn, 1980; Suresh & Srinivasan, 2007). Thus, bioaccessible capsaicinoids can be found as constituents of mixed micelles as well as free molecules dispersed in the aqueous fraction of the chyme. Suresh and Srinivasan (2007) demonstrated that the micellarization of CAP increased its incorporation into epithelial cells, but free CAP was also highly incorporated by cells. However, the lipophilic nature of capsaicinoids might cause, under some conditions, their entrapment into fat droplets, reducing their bioaccessibility and bioavailability. To date, the effect of fat presence and type on capsaicinoid bioaccessibility has not been tested, neither with pure nor dietary capsaicinoids. However, some studies collectively suggest that the presence of fat and emulsifiers (olive oil or Tween 80) improves the capsaicinoid bioaccessibility and bioavailability, as compared with that of aqueous dispersions of capsaicinoids (Donnerer et al., 1990; Kawada et al., 1984; Monsereenusorn, 1980; Suresh & Srinivasan, 2007).

Mexico is the second producer of green peppers worldwide. Jalapeño peppers are one the most cultivated, exported and consumed genotypes of pungent peppers in Mexico and are now important produce in the global market (Alvarez-Parrilla et al., 2011; Azcarate & Barringer, 2010; Ornelas-Paz et al., 2010). Thus, the objective of this study was to evaluate the effect of ripening, heat-processing style and freezing of Jalapeño peppers on the *in vitro* digestive stability and bioaccessibility of capsaicinoids in the presence and absence of two dietary fat types.

2. Materials and methods

2.1. Reagents and plant material

HPLC solvents, digestive enzymes, high purity standards of CAP and DHC (purity > 90%) and analytical grade reagents were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA), or Fisher Scientific Co. (Norcross, GA, USA), unless stated otherwise. Green and red Jalapeño peppers (cv. Marajá) were harvested from a commercial orchard in Chihuahua, Mexico. For the Caco-2 cell assays, Jalapeño peppers were purchased in a market in Columbus, OH. Only fruits without physical or biological damages were included in the study.

2.2. Treatments and in vitro digestion procedure

Fruits of each ripening stage were randomly distributed into 24 samples with 20 fruits each. Three fresh samples were boiled (94 °C/12.5 min) and another three samples were grilled (210 °C/13.2 min). Fifteen samples of fresh peppers were frozen using liquid N_2, packaged in polyethylene bags and stored at −20 °C in darkness. The remaining three samples were used as fresh raw controls. Fresh and cooked samples were immediately analyzed. Frozen samples were analyzed after 1, 2, 3, 4 and 6 months of storage. Each sample was homogenized to puree using a kitchen blender. Two grams of puree were subjected to gastric and small intestinal phases of digestion *in vitro*, as described by Victoria-Campos et al. (2013). Purees from fresh, boiled and grilled peppers were digested in the absence and the presence (120 μl) of either beef tallow (BT) or soybean oil (SO). Frozen peppers were digested without fat. The aqueous phase after small intestinal digestion was obtained by centrifugation (20,000*g*/15 min/4 °C) (Allegra 64R, Beckman Coulter Inc., Indianapolis, IN, USA) and filtration of the supernatant (0.22 μm, nylon membrane; Millipore Corp., Bedford, MA, USA). CAP and DHC were quantified in pepper purees, digestata and aqueous phases to determine the stability during digestion and the bioaccessibility of capsaicinoids. The digestive stability represented the fraction, expressed as a percentage, of capsaicinoids from pepper puree recovered after the digestion process (amount of capsaicinoids in digesta/amount of capsaicinoids in 2 g of pepper puree × 100). The bioaccessibility represented the fraction, expressed as a percentage, of capsaicinoids from pepper puree transferred to the aqueous phase during the digestion process (amount of capsaicinoids in the aqueous phase/amount of capsaicinoids in 2 g of pepper puree × 100). Pureed peppers were analyzed immediately for capsaicinoid content while digestata and aqueous phases were stored at −70 °C and analyzed within three days after digestion.

2.3. Capsaicinoid uptake by Caco-2 cell monolayers

Cultures of Caco-2 cells (HTB37, American Type Culture Collection; passage 29) were maintained (in 6-well) plates, as previously described (Ornelas-Paz et al., 2008), and used at 12 d after the monolayer was confluent. Filtered aqueous fractions were diluted 1:4 with basal DMEM (pH 6.5) to prepare the test medium for addition to monolayers (2 ml/well). Test medium also was incubated in cell-free wells to determine stability of capsaicinoids in the cell culture environment. Plates without and with cells were incubated for 4 h in a humidified atmosphere of 95% air and 5% CO_2. Integrity of cell monolayers was observed hourly by phase contrast microscopy to assess potential toxicity of the diluted aqueous fraction. Exposure did not cause changes in either cell morphology or adherence of the monolayer to the dish surface.