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Capsaicinoids and pungency in *Capsicum chinense* and *Capsicum baccatum* fruits[1]

Narda Gavilán Guillen[2], Richard Tito[3], Norma Gamarra Mendoza[2]

ABSTRACT

Capsicum chinense Jacq. and *C. baccatum* var. *pendulum* fruits are widely used in the food and processed food industry, in Peru, but their seeds and placentas are discarded as residues. This study aimed to quantify the proportion of edible (pericarp) and non-edible (seeds, placenta and interlocular septa) parts of the fruits, in market condition (semi-dried fruits of *C. chinense* and fresh fruits of *C. baccatum*), as well as to quantify the capsaicinoids and their pungency, in extracts of each fruit part previously dried. The pericarp represents 63 % and 85 % of the fruit, respectively for *C. chinense* and *C. baccatum*. The placenta stands for ~10 % of the fruit in both species, whereas, for the seeds, the index is 23 % in *C. chinense* and 5 % in *C. baccatum*. The content of capsaicinoids and pungency vary among the fruit parts and the species. High contents of capsaicinoids and pungency are found in non-edible parts of the fruit, mainly in the placenta (79 % in *C. chinense* and 51 % in *C. baccatum*). Regardless of the fruit part and species, the capsaicin was the major component of capsaicinoids (4,399 ug g^{-1} and 1,582 ug g^{-1} of the dry weight in *C. chinense* and *C. baccatum*, respectively), while dihydrocapsaicin and nordihydrocapsaicin reached a lower content. *C. chinense* contains more capsaicinoids and, thus, a much higher level of pungency than the *C. baccatum* fruits.

KEYWORDS: Capsaicin; dihydrocapsaicin; pepper.

RESUMO

Capsaicinoides e pungência em frutos de *Capsicum chinense* e *Capsicum baccatum*

Frutos de *Capsicum chinense* Jacq. e *C. baccatum* var. *pendulum* são amplamente utilizados na alimentação e na indústria de alimentos processados, no Peru, mas as sementes e placentas são descartadas. Objetivou-se quantificar a proporção de partes comestíveis (pericarpo) e não comestíveis (sementes, placenta e septos interloculares) dos frutos, em condições de mercado (frutos semissecos de pimenta *C. chinense* e frutos frescos de pimenta *C. baccatum*), bem como quantificar os capsaicinoides e sua pungência, em extrato de cada parte do fruto previamente seco. O pericarpo representa 63 % e 85 % do fruto, respectivamente para *C. chinense* e *C. baccatum*. A placenta responde por ~10 % do fruto em ambas as espécies, enquanto, para as sementes, o índice é de 23 % para *C. chinense* e de 5 % para *C. baccatum*. O conteúdo de capsaicinoides e a pungência variam entre as espécies e partes do fruto. Altos conteúdos de capsaicinoides e pungência são encontrados em partes não comestíveis do fruto, principalmente na placenta (79 % em *C. chinense* e 51 % em *C. baccatum*). Independentemente da parte do fruto e da espécie, a capsaicina foi o principal componente dos capsaicinoides (4.399 ug g^{-1} e 1.582 ug g^{-1} do peso seco em *C. chinense* e *C. baccatum*, respectivamente), enquanto a diidrocapsaicina e a nordi-idrocapsaicina atingiram menor concentração. *C. chinense* contém mais capsaicinoides e, portanto, nível muito mais alto de pungência que os frutos de *C. baccatum*.

PALAVRAS-CHAVE: Capsaicina; diidrocapsaicina; pimenta.

INTRODUCTION

Chili belongs to the *Capsicum* (Solanaceae) genus. The term capsicum derives from the Greek word “kapso”, which means “to bite”, thus referring to the pungency of the fruit (Maga & Todd 1975). The alkamide compound (bioactive agent) present in the fruits of all *Capsicum* species provides the organoleptic properties of pungency, being an intrinsic characteristic of this genus. Specifically, capsaicinoid compounds cause pungency. Among the capsaincinoid components, the most abundant, and responsible for approximately 90 % of the total pungency, are capsaicin (*trans*-8 methyl-*N*-vanillyl-6-nonenamide) and dihydrocapsaicin (8 methyl-*N*-vanillylnonanamide) (Li et al. 2009, Sganzerla et al. 2014). Additionally,

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2. Universidad Nacional del Centro del Perú, Facultad de Ingeniería de Industrias Alimentarias, Laboratorio de Biotecnología de Alimentos y Compuestos Bioactivos, Huancayo, Perú. *E-mail/ORCID*: nardis2112@hotmail.com/0000-0003-3922-0556, aunonegame2016@outlook.com/0000-0001-6559-6720.
3. Universidade Federal de Uberlândia, Instituto de Biologia, Uberlândia, MG, Brasil. *E-mail/ORCID*: rtitoleon@gmail.com/0000-0002-4184-5654.

chili fruits contain other capsaicinoid components, such as nordihydrocapsaicin, norcapsaicin, homocapsaicin I, homodihydrocapsaicin I, homocapsaicin II, homodihydrocapsaicin II and nonivamide (Batchelor & Jones 2000, Barbero et al. 2008).

Capsaicinoids are synthesized and accumulated in the epidermal cell of the placenta, but they are also found in the pericarp, seeds and other vegetative structures, such as leaves and stem (Fattorusso & Taglialatela-Scafati 2008, Barbero et al. 2014). The synthesis of capsaicinoids occurs in the early stages of fruit formation, between 20 and 50 days after the anthesis, and the amount of capsaicinoids increases with fruit maturation (Contreras-padilla & Yahia 1998). The biosynthesis of capsaicinoids depends on genotype, fruit maturity and environmental factors, such as solar radiation and water availability (Zewdie & Bosland 2000, Topuz & Ozdemir 2007, Gangadhar et al. 2012, Rahman & Inden 2012).

Capsaicinoids have several properties and applications, including the chemopreventive, anti-mutagenic, anti-tumor, anti-inflammatory, antioxidant, analgesics, body temperature regulator, fungicide, bactericide, nematicide and insecticide ones, as well as insect repellent (Jones et al. 1997, Kurita et al. 2002, Athanasiou et al. 2007, Meghvansi et al. 2010). Therefore, the potential use of chili capsaicinoids in the pharmaceutical, agronomic and veterinary industries make them very interesting compounds to be studied.

Peru is the origin center of many species and varieties of chili, as well as one of the world's largest producers. For instance, in 2015, the chili production reached 45,470 t (Gómez 2016). The most cultivated and commercialized Peruvian native chilies are *Capsicum chinense* Jacq. [locally known as *panca* chili. In Mexico, it is also known as *habanero* chili, although the color varies (Figure 1a)] and *C. baccatum* var. *pendulum* [locally known as *orange* chili (Figure 1b)] (Peru 2014). The fruits of these two chili species are widely used in the food and food processing industry, and large quantities of their seeds and placentas (residues) are discarded.

This study aimed to quantify the proportion of edible (pericarp) and non-edible (seeds, placenta and interlocular septa) parts of fruits of these two economically important chili species, as well as to quantify the amount of capsaicinoids and their pungency from extracts of each part of the fruit, in order to determine in which of these parts the highest concentration is found. The quantification of capsaicinoids in the residues (i.e., placenta and seeds) can be useful in suggesting the potential use of those discarded parts for valuable purposes in medicine or agriculture.

MATERIAL AND METHODS

The study was performed from March 2016 to March 2017. A total of 5 kg of *Capsicum chinense*

Photos: Norma Gamarra Mendoza

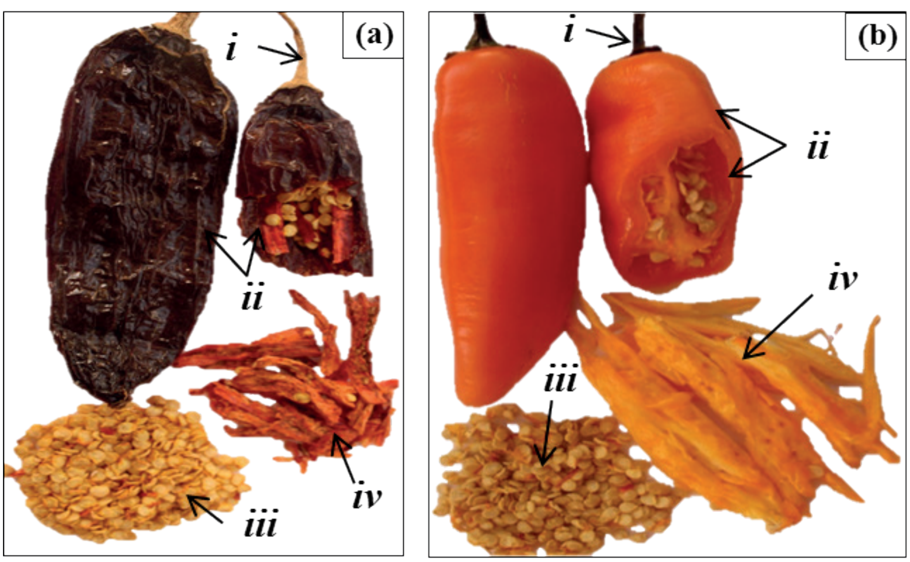

Figure 1. Fruits of *Capsicum chinense* (a) and *C. baccatum* (b), showing the different fruit parts/structures: peduncle (*i*), pericarp (*ii*), seed (*iii*) and placenta (*iv*).

Jacq. and *C. baccatum* var. *pendulum* fruits were obtained from the Sazón Lopeza industry and from the largest municipal market of the city of Huancayo, in Peru. From this set of fruits, 20 fruits were randomly sampled for each species. Fruits of *C. chinense* were semi-dried and fruits of *C. baccatum* were fresh (Figure 1), conditions in which they are commonly used and sold. Sampled fruits were transported to the laboratory, in order to measure the fruits and determine their content of capsaicinoids.

The sizes of the fruits were measured and the pericarp, placenta and interlocular septa (hereafter referred to simply as "placenta") and seeds were separated (Figure 1), weighed and dehydrated at 45 ± 3 ºC. The samples were dried in a hot air convection tray dryer, at an inflow speed of 4.83 m s^{-1}, for 7 h, reaching a humidity of 10 ± 2 %. For each dry sample (i.e., the pericarp, placenta and seeds) and species separately, dry samples were crushed and sieved, in order to obtain suitable and uniform particle sizes (0.425-1.000 mm). The extraction of capsaicinoids was performed using the standardized procedures in the literature (Koleva-Gudeva et al. 2013a). Specifically, 8 g of crushed sample were dissolved in 30 mL of ethanol (99 %), under a stirring speed of 270 rpm, at 60 ºC, for 25 h, time enough to solubilize and increase the extraction, due to a greater distribution of the solvent, homogeneous temperature and close contact between the solute and solvent (Fernández Barbero 2007). The extract was cooled, filtered and evaporated using a rotary evaporator (R-200-R1 BÜCHI), at 40 ºC and 500 mm Hg, for 15 min.

The dried extract of each part of the fruits was diluted in 25 mL HPLC-grade methanol, and then 2 mL of methanolic extract were filtered in a polyfluoride vinylidene acrodisc membrane (PVDF) of 0.45 µm, in a vial. In three samples (replicates), the capsaicinoids were quantified by high-performance liquid chromatograph (HPLC, Shimadzu UFLC model, Tokyo, Japan), with a pinnacle II column (C18, 250 mm x 5 µm, 4.6 µm). The capsaicinoids were separated at 30 ºC, with a flow speed of the mobile phase of 1.5 mL min^{-1}. The mobile phase was prepared with water at 1 % of acetic acid and acetonitrile (50:50 v/v) mix. Elution was performed under isocratic conditions for 20 min. The reading was performed at 280 nm. For this, the calibration curve was elaborated by previously using each of the standards of capsaicin, dihydrocapsaicin and nordihydrocapsaicin, in concentrations of 0.02 mg mL^{-1}, 0.06 mg mL^{-1}, 0.1 mg mL^{-1}, 0.14 mg mL^{-1} and 0.18 mg mL^{-1} for each one. The chromatographic profiles of the standard solutions were compared with those of the sample under study, considering the retention time, spectrum, area and height. In other words, the capsaicinoids were identified and quantified using the linear equation of the calibration curve of capsaicin, dihydrocapsaicin and nordihydrocapsaicin standards.

Pungency was determined in Scoville Units (SHU), following the procedures commonly used in the literature (Helrich 1990, Sanatombi & Sharma 2008). The particular capsaicinoid content of the chili dry weight was multiplied by the coefficient corresponding to the pure pungency threshold. That is, 16.1 million for capsaicin, 15 million for dihydrocapsaicin and 9.3 million for nordihydrocapsaicin.

The concentration of each capsaicinoid was quantified using the linear regression between the areas of each chromatogram and the concentration of the capsaicinoid standards. The difference in the capsaicinoids content and pungency between the species (*C. chinense* and *C. baccatum*) and the parts of the fruits (placenta, seed and pericarp) were analyzed using a two-way analysis of variance. For multiple comparisons, the Tukey test was applied. Finally, a simple correlation test was performed to test the association between the capsaicinoids contents and the pungency. The assumptions of the parametric tests were cheked through the residual plots. The statistical analyses were carried out by using the Systat v. 10.2 software.

RESULTS AND DISCUSSION

The edible (pericarp) and non-edible (placenta, seeds and peduncles) parts of the chili fruits are commonly separated for direct consumption or for the food processing industry. The pericarp represents the largest proportion of the fruit: 62.5 ± 1 % in *C. chinense* and 85.1 ± 1.3 % in *C. baccatum* (recalling: semi-dried fruits of *C. chinense* and fresh fruits of *C. baccatum*). Among the non-edible parts of the fruit, the placenta was ~10 % in both species, whereas the *C. chinense* seeds were 22.5 ± 0.9 % and the *C. baccatum* seeds were 4.6 ± 0.9 %. Therefore, the main discarded structures (i.e., placentas and seeds) that contain capsaicinoids can make up to ~33 % of the fruit. The proportions of the fruit parts found here were similar to other chili species, independently of the fruit size (Buczkowska et al. 2016). Fruits of the *Capsicum* species differ widely in size and shape, but our focal species showed approximately homogeneous characteristics, cylindrical-conical elongated, with average dimensions of 11.3 ± 0.76 cm long and 3.4 ± 0.3 cm of diameter. The fruits have from two to four cavities, between the placenta and the interlocular septa (Figure 1). Fruits of *C. chinense* have an intense red-brown color and are sold in the domestic market as a whole dried chili, whereas fruits of *C. baccatum* are sold in fresh conditions, processed paste and dehydrated powder. Both chili species are in great demand, due to their pungent taste and color, for the preparation of culinary foods (Gómez 2016).

The retention times of the chromatograms and their corresponding spectra were compared to identify the capsaicinoids (Figure 2). The absorption spectrum in the ultraviolet region was detected at 280 nm and, under these conditions, the chromatograms were separated without the presence of interfering substances. Most capsaicinoids exhibit their absorption spectra between 200 nm and 350 nm (González-Zamora et al. 2013). The chromatograms of capsaicin and dihydrocapsaicin from the different fruit parts of both chili species showed an area under the curve in milliunits of absorbance (mAU) and a higher height percentage, which means that these compounds predominate in our focal species. Therefore, the linear regression between the areas of each chromatogram and the concentration (mg mL^{-1}) of the capsaicinoid standard resulted in a

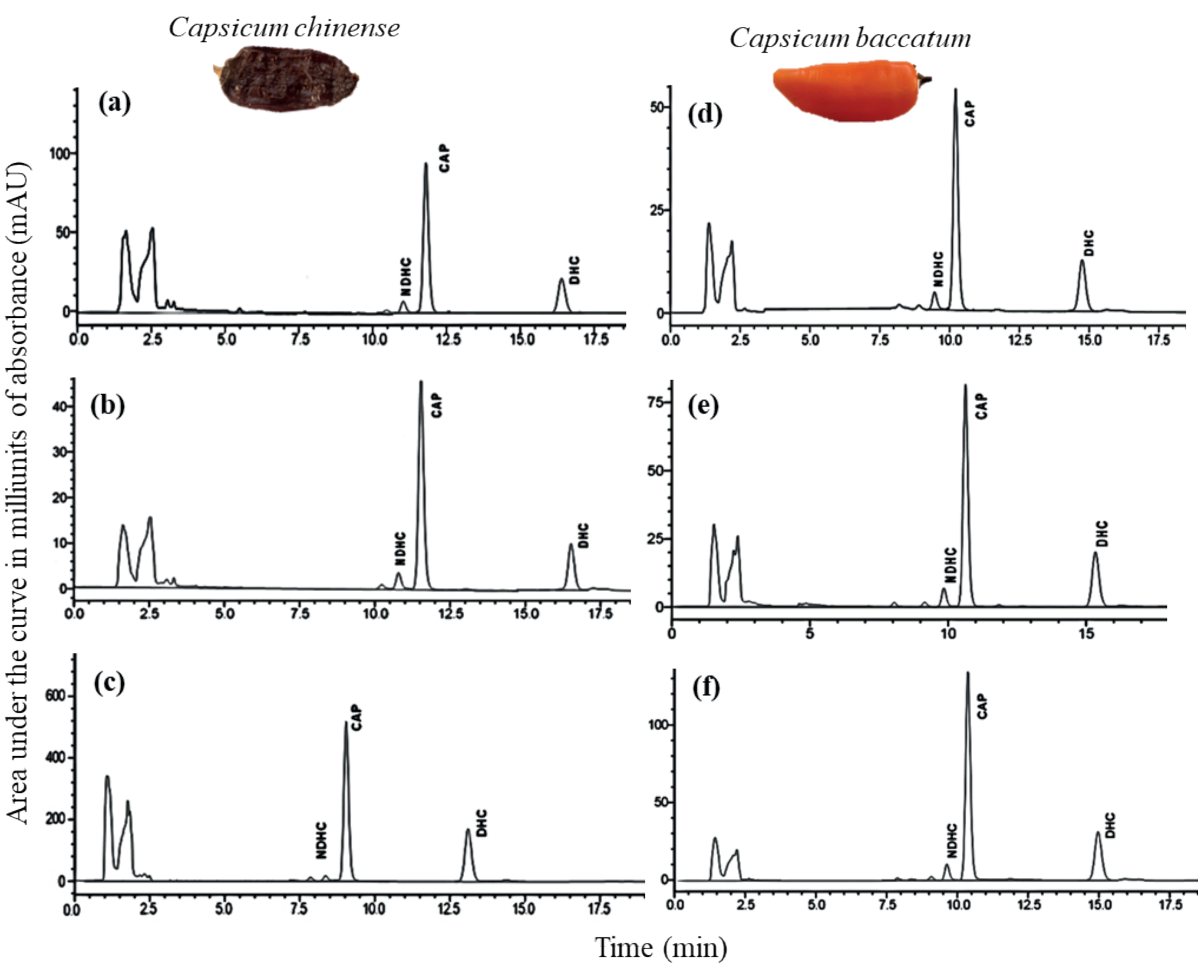

Figure 2. Chromatogram showing the capsaicinoids in the pericarp (a), seeds (b) and placenta (c) of *Capsicum chinense* fruits; and in the pericarp (d), seeds (e) and placenta (f) of *C. baccatum* fruits. NDH: nordihydrocapsaicin; CAP: capsaicin; DHC: dihydrocapsaicin. Note the high absorbance value (mAU) for the placenta of *C. chinense* and *C. baccatum* fruits (c), indicating the high concentration of capsaicin, differently from the others.

high percentage of association ($R^2 > 99$ %) for each capsaicinoid concentration.

The capsaicinoids content varied among the species ($F_{1,12} = 1934$, $p < 0.001$) and the fruit parts ($F_{2,12} = 2779$, $p < 0.001$) (Figure 3a). The content of capsaicinoid and pungency were markedly higher in the placenta than in the pericarp or seeds, both in *C. baccatum* ($F_{2,6} = 14080$, $p < 0.001$) and mainly in *C. chinense* ($F_{2,6} = 2156$, $p < 0.001$) (Figure 3a). The greater amount of capsaicinoids in the placenta is probably related to the site of biosynthesis that is produced mainly in this structure of the fruit (Nowaczyk et al. 2006a and 2006b, Pandhair & Sharma 2008), and from where it is transferred to the other fruit structures (Buczkowska et al. 2016). Several previous studies also have shown this difference of a higher capsaicinoid content in the placenta of fruits of different chili species (Canto-Flick et al. 2008, Pandhair & Sharma 2008). For instance, the amount of capsaicinoids in the placenta of *Capsicum annum* fruits is 10 times higher than in the pericarp and seeds (Pandhair & Sharma 2008).

The variation of capsaicinoid content among chili species is expected, due to the intrinsic biological characteristics of each species. In the present study, *C. chinense* contains more capsaicinoids than *C. baccatum*, a fact that is reflected in the concentration at least four times higher in the placenta (Figure 3a). The interspecific variations are attributed to the genotype and the variability of abiotic conditions in which *Capsicum* species grows, as temperature, humidity, nutrient and other factors that influence positively or negatively the capsaicinoids biosynthesis (Contreras-Padilla & Yahia 1998, Gnayfeed et al. 2001, Sung et al. 2005, Canto-Flick et al. 2008, Mueller-Seitz et al. 2008, Rahman & Inden 2012, Giuffrida et al. 2013, González-Zamora et al. 2013).

Independently of the fruit parts and the chili species, the capsaicin concentration predominates (74-76 %), while the dihydrocapsaicin and nordihydrocapsaicin contents reach lower percentages (Table 1). As shown here and in other studies, the pungency is highly and directly related to the amount of capsaisinoids, more specifically to the amount of capsaicin and dihydrocapsaicin, which are responsible for the level of the peppery taste of chilies (Li et al. 2009, Sganzerla et al. 2014). Therefore, a similar pattern of variation in the content of these compounds reflected the pungency variations among the species ($F_{1,12} = 1968$, $p < 0.001$) and the fruit parts ($F_{2,12} = 2871$, $p < 0.001$) (Figure 3b). That is, the placenta presented a high pungency, if compared to the pericarp (the edible part) and seeds, with a lower pungency for both *C. chinense* ($F_{2,6} = 2222$, $p < 0.001$) and *C. baccatum* ($F_{2,6} = 14756$, $p < 0.001$) (Figure 3b). Nevertheless, including all parts, *C. chinense* fruits presented a pungency at least four times greater than for *C. baccatum* (Figure 3b). In fact, *C. chinense* is recognized as the most pungent species within the domesticated *Capsicum* species (Canto-Flick et al. 2008). According to the pungency in Scoville (SHU)

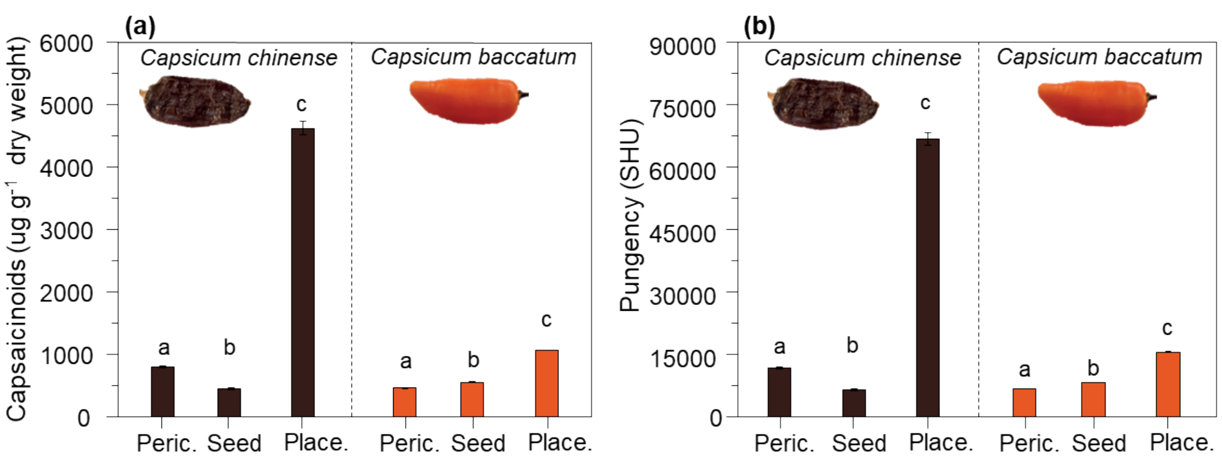

Figure 3. Averages (± SE) of total capsaicinoides (a) and pungency (b) (expressed in Scoville Units - SHU) of *Capsicum chinense* and *C. baccatum* edible [pericarp (peric.)] and non-edible [seed and placenta (place.)] parts. Different letters above the SE bars indicate a significant different ($p < 0.05$) concentration among the fruit parts.

Table 1. Amount of total capsaicinoids (mean ± SD, n = 3) in the pericarp, seeds and placenta of *Capsicum chinense* and *C. baccatum*. Different letters indicate that there are significantly different concentrations among the fruit parts ($p \leq 0.05$).

Species/fruit part	Capsaicin	Dihydrocapsaicin	Nordihydrocapsaicin
	ug g^{-1} of dry weight		
Capsicum chinense			
Pericarp	610.9 ± 15.6 a	167.6 ± 2.2 a	26.5 ± 1.9 a
Seed	336.6 ± 15.9 b	95.1 ± 6.8 b	17.8 ± 1.8 a
Placenta	3,451.6 ± 101.8 c	1,008.7 ± 36.5 c	162.7 ± 11.1 b
Total	4,399.1 ± 87.4	1,271.4 ± 34.8	207.0 ± 9.2
Capsicum baccatum			
Pericarp	355.7 ± 4 a	78.6 ± 2 a	23.9 ± 1 a
Seed	423.0 ± 2.5 b	103.8 ± 0.8 b	29.1 ± 0.3 b
Placenta	802.8 ± 3.4 c	198.8 ± 0.9 c	60.6 ± 1.9 c
Total	1,581.6 ± 9.2	381.2 ± 3.2	113.5 ± 0.8

categories, fruits of *C. chinense* are classified as very pungent (> 80,000 SHU), while *C. baccatum* fruits are considered mildly pungent (Weiss 2002). The pungency levels are also supported by the corresponding percentage of the capsaicin content in the fruits of chili species that were studied here. The percentage content of capsaicinoids reached 0.6 % and 0.2 % of dry weight for the *C. chinense* and *C. baccatum* fruits, respectively. A previous study (Yao et al. 1994) defined that the mild peppery varieties contain capsaicinoids concentrations in the range of 0.01-0.3 %, while very peppery varieties contain more than 0.3 % in capsaicinoids.

Somes studies have shown that pests and diseases will likely become a major threat to the tropical agriculture under climate change conditions (Bebber et al. 2014, Tito et al. 2018). Therefore, developing a suitable environmentally friendly control management is essential, and the use of capsaicinoids may be helpful for this purpose. There are several evidences that these compounds may be used as bio-insecticides to control several pests that affect different crops. For instance, capsaicin is an effective biopesticide against *Myzus persicae* and *Leptinotarsa decemlineata* (Maliszewska & Tęgowska 2012, Koleva-Gudeva et al. 2013b).

The high concentration of capsaicinoids in non-edible parts of chili fruits also highlights the potential use of these materials that will be low cost raw materials. Here, we show that discarded structures (placenta and seeds) reach up to 33 % and, considering the total chili production in Peru for 2015 [45,470 t (Gómez 2016)], more than 14,000 t must have been discarded.

CONCLUSIONS

1. The pericarp represents 63 % and 85 % of the *Capsicum chinense* and *C. baccatum* fruits, respectively, while the placenta stands for 10 % of the fruit in both species, whereas the seeds reach 23 % and 5 %, respectively in *C. chinense* and *C. baccatum*;
2. For both *C. chinense* and *C. baccatum*, the highest capsaicinoids concentrations are found in the placenta than in the pericarp or seeds;
3. Among the capsaicinoid components, capsaicin is the most abundant, followed by dihydrocapsaicin and nordihydrocapsaicin;
4. The pungency levels are directly related to the capsaicinoids content, mainly to the capsaicin concentration.

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