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Department of Sustainable Technologies

Influence of drying on final content of capsaicinoids in selected varieties of chili peppers

M.Sc. Thesis

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DECLARATION

I, Cesar Orlando Fonseca Cruz, hereby declare that this thesis, submitted in partial fulfilment of requirements for the master degree in Faculty of Tropical AgriSciences of the Czech University of Life Sciences Prague, is wholly my own work written exclusively with the use of the quoted sources.

In Prague on 27/04/2017

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Cesar Orlando Fonseca Cruz

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ABSTRACT

Chili peppers from different *Capsicum* species are used worldwide in food preparations for their pungent flavour. Additionally, their nutritional value and health promoting substances has arisen the interest of food and pharmacological industries. The high demand for chili peppers makes necessary the use of drying for their preservation. Nonetheless, the drying process can influence the flavour and nutritional qualities of chilies by affecting the concentration of different substances; among them those responsible for pungency, called capsaicinoids. This thesis investigates the influence of drying on the final content of capsaicinoids in selected *Capsicum* species. Fully ripen fruits of 11 different cultivars, not previously studied, were analysed. Part of the samples were evaluated as fresh and the rest was prepared in three different ways (whole, cut in half, mashed) and dried separately at 40 and 60 °C. Capsaicin and Dihydrocapsaicin were quantified by means of GC-MS to estimate the pungency in both dried and fresh peppers. Pungency values for fresh peppers ranged between 90,927 and 381,639 SHU. The influence of drying on capsaicinoids changed diversely depending on the variety of chili pepper. Mashed pre-treatment in combination with 60 °C temperatures showed significant reductions in capsaicinoids contents, as much as 59 %. Meanwhile, at 40 °C significant improvements were evidenced for whole and half-piece preparations, up to 43 % and 74 %, respectively. Different behaviours between individual capsaicinoids in relation to the different drying conditions were observed as well.

Keywords: Capsaicin, Chili pepper, Dihydrocapsaicin, Drying, Gas chromatography-mass spectrometry, Pungency.

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1 INTRODUCTION

Chili peppers, fruits of plants of the *Capsicum* genus, are used as one of the main spices and ingredients in food preparations worldwide due to its intense flavour, aroma and colour characteristics (Cisneros-Pineda et al., 2007).Supplementary to its organoleptic properties, chilies are highly valued for providing nutritional and health promoting compounds, such as antioxidants (vitamins C and A) and mineral elements. Furthermore, chilies are currently utilised in a wide range of applications including alternative medical treatments for inflammation, diabetes and low back pain (Aggarwal and Kunnumakkara, 2009) and as bio insecticide.

Capsaicinoids are the main responsible for the pungent sensation produced by chili peppers. The quantification of these substances through modern techniques is of great value to consumers and industries as it allows to identify the precise level of pungency in the different *Capsicum* fruits and chilli pepper products. As the demand for chili peppers grows in the household and industry sectors, suitable preservation methods are necessary to ensure a shelf-stable product with the proper quality characteristics. Drying is the most common method used for food preservation, it favours the long shelf-life of chili peppers and eases handling operations of food processors.

Nevertheless, the drying process causes changes in food properties including loss of colour, nutritious and flavour characteristics which may affect the judgement of consumers. The economic importance of chili peppers and the lack of investigations regarding the influence of drying on the pungency are the main drivers of this study.

2 LITERATURE REVIEW

2.1 ORIGIN AND DISPERSION OF CHILI PEPPERS

Capsicum, member of the Solanaceae (nightshade) family and with approximately 35 species and more than 2000 cultivars, is native to tropical Americas and its origin can be tracked back to an area along the Andes of western to north-western South America. The expansion of the genus has followed a clockwise direction around the Amazon basin, towards central and south-eastern Brazil, then back to western South America, and finally northwards to Central America (Tewksbury et al., 2006; Carrizo García et al., 2016) .

Despite being domesticated more than six thousand years ago, chilies were only introduced worldwide with the discovery of America, by late 15th century; brought to Europe as a replacement for the expensive black pepper (*Piper nigrum* L.), imported from Asia in that time; and incorporated to Africa and Asia by Portuguese traders (NUEZ et al., 1996 by Domenico, 2011)**.**

Out of the list of *Capsicum* cultivars only 5 species have been domesticated: *C. annuum*, most widely grown today, both in the Americas and world-wide; *C. chinense*, with rising popularity attributed to its high pungency and distinct flavour profile; *C. frutescens*, also cultivated in Africa and Asia as a spice crop for consumption and its oleoresins; *C. baccatum* and *C. pubescens*, both prevalently confined to the Andean countries (Pickersgill, 1997). Nonetheless, there is much confusion on the botanical classification of chili peppers as their size, colour, shape and pungency are subjected in great measure to the environmental conditions and the development of varietal cultivars (Reineccius, 2013).

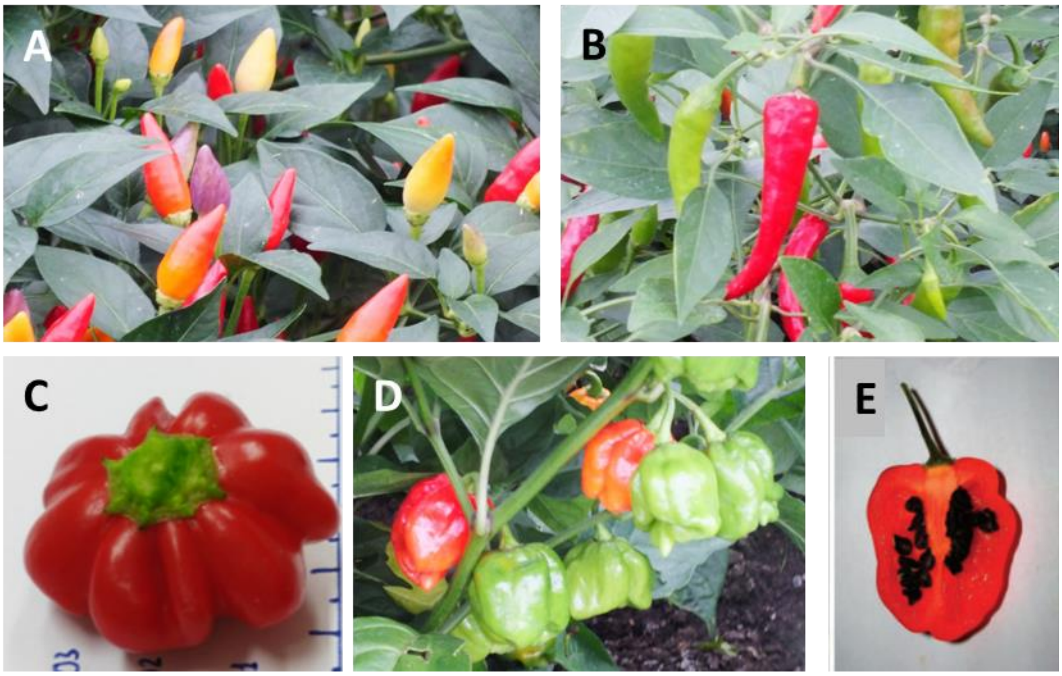


Figure 1 Fruits of C. frutescens Twilight (A), C. annuum L. Freuzr Werh (B), C. baccatum Brazilian Starfish (C), C. chinense Jacq. Scotch Bonet, and C. pubescens (E). Photos by D. Claramount (A,B,C,D). Picture (E) taken from Carrizo García et al. (2016).

2.2 ECONOMIC IMPORTANCE OF CHILLI PEPPERS

Chili pepper is one of the most important horticultural crops nowadays. It is highly versatile as it can be used fresh, both green and ripe to impart pungency to the food or as a vegetable; and as a condiment in various processed forms including pastes, pickles or dried powder (Cankaya et al., 2017).

Globally, 31,177,539 tonnes of fresh and 3,618,392 tonnes of dried chilies were produced in the year 2013; the greatest portion produced by Asia (64.6 %), followed by Africa (24 %), the Americas (6.8 %), Europe (4.5 %) and Oceania (0.1 %). In terms of exports, 4,959,269 USD of fresh and 1,231,246 USD of dried peppers were dealt in 2013; the largest share was exported by China, Mexico, Turkey, Indonesia and Spain; and India, China, Thailand and Peru, respectively for fresh and dried chilies (FAO, 2017)

In recent years, the interest in chili peppers has remarkably enlarged; its demand rose 28.45 % between the years 2003 and 2013 (FAO, 2017). This increase is partly because of the widespread multiculturalism, popularity of ethnic restaurants, and the increased demand for vegetables associated with high nutritional value and low calorie content (Orellana-Escobedo et al., 2013).

2.3 NUTRITIONAL VALUE

Chili peppers are an important source of protein, carbohydrate, lipids, mineral salts, vitamins A and B complex (thiamine, riboflavin, niacin, B6 and folic acid), water and fibres necessary for the normal functioning of human and animal organism (Domenico, 2011; Rêgo et al., 2012; Moresco, 2013).

Proximate nutritional contribution of raw ripen chili peppers, per 100 g serving, including energy, protein, fat, carbohydrates, dietary fibre, and relevant mineral and vitamin values, are shown in Table 1 in accordance with the data published by the Agricultural Research Service of the United States Department of Agriculture. Nonetheless, alternative researches point out that dietary composition of peppers may fluctuate enormously among different *Capsicum* varieties (Rêgo et al., 2012).

In addition, chili peppers comprise considerable amounts of carotenoids, bioactive compounds with outstanding antioxidant properties and responsible for the colour in fruits and vegetables. Carotenoids identified include capsanthin, capsolutein, capsorubin, cryptoxanthin, lutein, luteoxanthin, mutatoxanthin, violaxanthin, α-carotene, β-Carotene and β-cryptoxanthin (Antonious et al., 2009; Giuffrida et al., 2013; Moresco, 2013; Mokhtar et al., 2016).

Chilies also contain important amounts of ascorbic acid (vitamin C); fruits in advanced ripening stages usually contain higher values (Nagy et al., 2015). Moreover, accessions of *C. chinense* generally contain higher amounts of this vitamin, in some cases even higher than the current daily recommendation for human diet (Moresco, 2013; Teodoro et al., 2013). Chili peppers comprise numerous bioactive substances of great importance, but perhaps the most relevant among them are those responsible for their pungency.

Table 1 Proximate nutritional contribution of chili peppers. Data published by the Agricultural Research Service of the United States Department of Agriculture (USDA, 2016).

Nutrient	Unit	Value per 100g
Proximate		
Water	g	88.02
Energy	kcal	40
Energy	kJ	166
Protein	g	1.87
Total lipid (fat)	g	0.44
Ash	g	0.87
Carbohydrate, by difference	g	8.81
Fibre, total dietary	g	1.5
Sugars, total	g	5.3
Minerals		
Calcium, Ca	mg	14
Iron, Fe	mg	1.03
Magnesium, Mg	mg	23
Phosphorus, P	mg	43
Potassium, K	mg	322
Sodium, Na	mg	9
Zinc, Zn	mg	0.26
Copper, Cu	mg	0.129
Manganese, Mn	mg	0.187
Selenium, Se	µg	0.5
Vitamins		
Vitamin C, total ascorbic acid	mg	143.7
Thiamine	mg	0.072
Riboflavin	mg	0.086
Niacin	mg	1.244
Pantothenic acid	mg	0.201
Vitamin B-6	mg	0.506
Choline, total	mg	10.9
Vitamin A, RAE	µg	48
Carotene, beta	µg	534
Carotene, alpha	µg	36
Cryptoxanthin, beta	µg	40
Vitamin A, IU	IU	952
Vitamin E (alpha-tocopherol)	mg	0.69

2.4 PUNGENT PRINCIPLE OF CHILI PEPPERS

Fruits of *Capsicum* species accumulate and secrete severely pungent materials; this hot and irritating effect is caused by a group of compounds called capsaicin and its analogues. Capsaicinoids, as they are called as a group and with a general structure [N-(4-hydroxy-3-methoxybenzyl)alkyl- amides] constituted by nitrogen, oxygen and hydrogen atoms, are recognized among the members of Alkaloids (Suzuki and Iwai, 1984).

2.4.1 Formation and accumulation of capsaicinoids

Capsaicinoids are distributed differently along the peppers. Placental and dissepiment tissues contain the greatest amount of capsaicinoids; as much as ten times compared to the content in seeds and pericarp (Kozukue et al., 2005; Cisneros-Pineda et al., 2007). Further investigations, by means of electron-microscopy, have determined the intracellular location of capsaicinoids in the vesicles or vacuole-like subcellular organs of the epidermal cells of placenta, isolated from other subcellular organs (Suzuki and Iwai, 1984).

Figure 2 Inner structure of Capsicum fruits. Photo produced for this study.

2.4.1.1 Factors affecting formation and accumulation of capsaicinoids

Biological factors, like maturity stage of fruits and type of cultivar, have a great effect on the accumulation of capsaicinoids in *Capsicum* fruits. Capsaicin concentration increases gradually during fruit development reaching maximum levels at 40 to 50 days

(De Lourdes Reyes-Escogido et al., 2011); capsaicinoid content of fully-ripen chilies may range from 1.5-fold to 4.5-fold compared to immature peppers (Bae et al., 2014).

In the same way, environmental conditions and agronomic practices interact with genetics and other factors to alter the bioactive properties of plant fruitage. Accumulation of capsaicinoids in fruits can be importantly affected by temperature, light, elevation, and nutrient availability (Suzuki and Iwai, 1984). Drought stress is well recognized to influence the concentration of capsaicinoids; reduction of water application by 25 %, 50 %, and 75 % can cause an enormous increase (113 % to 721 %) on the capsaicinoid content of low and medium pungency cultivars at 10, 20, and 30 days after flowering (Phimchan et al, 2012).

Likewise, different cultivars raised in various locations have shown a consistent increment in capsaicinoids production as elevation increases, but a negative correlation regarding temperature and solar radiation (Gurung et al., 2011). The fact that non-pungent and relatively low-pungent plants dominate *Capsicum* populations in low-elevations, and pungent plants abound as altitude increases, suggests a correlation between elevation and capsaicinoid production (Tewksbury et al., 2006).

2.4.1.2 Biological significance

It is difficult to state a concrete reason for the accumulation of these pungent compounds in *Capsicum* fruits. Several theories speculate that capsaicinoids are just a waste product without any biological significance whereas others imply that its purpose is to repel invertebrate predators or even to protect seeds from fungal and bacterial decay (Tewksbury et al., 2006).

Antimicrobial of effect of *C. frutescens* extracts has been reported against gram positive bacteria (*B. cereus),* gram negative bacteria (*E. coli* and *P. aeruginosa)* and two fungi (*C. albicans* and *C. krusei*)(Gurnani et al., 2016). Besides, antibacterial and anti-virulence activity of sub lethal capsaicin concentrations against *Streptococcus pyogenes* has also been described, achieving significant increase in biofilm production, and reduction in cell-invasiveness and haemolytic activity (Facinelli, 2015). Notwithstanding the several discoveries, there is no consensus in the matter.

2.4.2 Chemical composition

The pungent principle of chilli peppers began to be studied as early as 1810; by 1876 Tresh isolated the pungent substance in crystalline form and name it capsaicin; after vigorous studies, between 1910 and 1923, Nelson and Dawson established the chemical structure of ‘capsaicin’ as the vanillylamide of 8-methylnon-6- enoic acid(De Lourdes Reyes-Escogido et al., 2011); in 1955 Crombie ef al. confirmed the chemical structure of ‘capsaicin’ as N-(4-hydroxy-3- methoxybenzyl)-methylnon-6-trans-enamide adding little changes previously proposed by Lapworth and Role.

Nonetheless, the task of completing the capsaicin chemical formula was not over yet. In 1985, Kosuge and his collaborators, by means of paper chromatography and subsequent colorimetric determination, reported that the chemically ‘pure capsaicin’ was a mixture of two closely related compounds in a ratio 2.1:1 (Suzuki and Iwai, 1984); the major compound was finally identified as capsaicin and its minor analogue as dihydrocapsaicin.

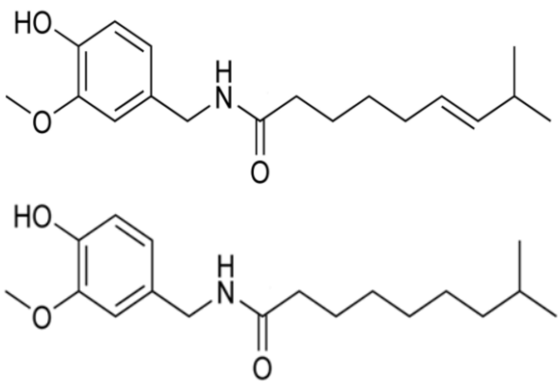


Figure 3 Structure of Capsaicin (top) and Dihydrocapsaicin (bottom) (Usman et al., 2014).

Capsaicin ((E)-N-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methylnon-6-enamide) is identified as the primary pungent principle in *Capsicum* fruits. It is a crystalline, lipophilic, colourless and odourless alkaloid with the molecular formula $C_{18}H_{27}NO_3$. Its molecular weight is 305.418 g/mol; boiling point 210-220 °C, melting point 65 °C, and vapour pressure 1.32×10^{-8} mm Hg (at 25 °C); and it is soluble in water (28.93 mg/L at 25 °C), in petroleum ether, and freely soluble in alcohol, ether, benzene and slightly soluble in carbon disulphide and hydrochloric acid (NCBI, 2017a)

Dihydrocapsaicin N-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methyl-nonanamide) is a lipophilic, colourless, odourless, crystalline to waxy alkaloid with the molecular formula $C_{18}H_{29}NO_3$. Its molecular weight is 307.434 g/mol; its melting point 65.5-65.8 °C; and it is soluble in dimethyl sulfoxide and 100 % ethanol (NCBI, 2017b).

Capsaicin and dihydrocapsaicin are the most potent compounds accounting for approximately 90 % of capsaicinoids in chili peppers. Traces of other capsaicinoids like nordihydrocapsaicin, nonivamide, homocapsaicin-I, homocapsaicin-II, homodihydrocapsaicin-I, homodihydrocapsaicin-II have been also identified (Kozukue et al., 2005; Barbero et al. 2008; Gahungu et al, 2011; Kollmannsberger et al., 2011; Daood et al., 2015).

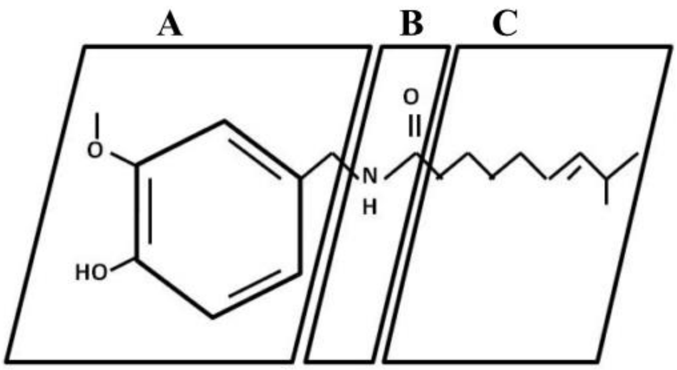


Figure 4 Regions of capsaicin molecule (De Lourdes Reyes-Escogido et al., 2011).

The capsaicin molecule can be represented by a combination of three different structures that perform specific functions: A (aromatic ring), B (amide bond) and C (hydrophobic side chain). The more than 20 identified capsaicinoids are structurally alike in the first two regions but differ in nature of the lateral chain of fatty acids (C), which ranges from 9 to 11 carbons long with a variable number of double bonds located in different positions along the chain (De Lourdes Reyes-Escogido et al., 2011).

2.4.3 Applications of Capsaicinoids

Recent discoveries on antimicrobial and anticarcinogenic properties of capsaicinoids has driven the attention of both the food industry and pharmacology (De Lourdes Reyes-Escogido et al., 2011). Investigation on health benefits in daily ingestion of chili comprise a positive relation to handgrip strength in an adult population (Wu et al., 2016), and the reduction in the hazard of death from heart disease and stroke (Chopan and Littenberg, 2017).

Added to the huge variety of chili processed products, applications in the food industry have tested the inhibitory profiles of capsaicin on the formation of mutagenic and carcinogenic compounds produced during high-temperature processing of protein-rich foods. Administration of 2 mg of capsaicin suppressed the generation of total Heterocyclic amines and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) concentrations in 80 % and 98 %, respectively (Zeng et al., 2017).

Moreover, pharmaceutical applications of capsaicin and analogues include: topical formulations in the management of neuropathic and osteoarthritis pain, capsaicin-based therapies for pain control, intranasal capsaicin in the treatment of nonallergic rhinitis, anti-obesity drug, potential antitumor effects, role in dermatology, use of vanilloids in urologic disorders (Mózsik et al., 2009; I. Nagy et al., 2014), and gastroprotective effect (De Lourdes Reyes-Escogido et al., 2011; Dömötör, 2014) .

2.4.4 Analysis of capsaicinoids

The use of analytical methods is necessary to determine the level of pungency in chili peppers in a reliable way. In the early years of capsaicinoid analysis, chemical essay procedures based in colorimetry and spectrometry, and sensory evaluation (Scoville organoleptic test) were employed (Suzuki and Iwai, 1984). Nowadays modern and more suitable techniques have been developed to have reliable reproducible results.

Ultrasound (Barbero et al., 2008), solid phase micro-extraction (Peña-Alvarez et al., 2009) or enzymatic pre-treatments (Salgado-Roman et al., 2008;Mokhtar et al., 2016) are used to increase efficiency in capsaicinoid extraction. In the same way, chromatographic methods like gas chromatography (GC) and high performance liquid chromatography (HPLC) enables capsaicinoid analyses to be more accurate, sensitive, and time efficient.

Further investigations regarding performance include HPLC-fluorescence detection (Barbero et al., 2008), HPLC-MS (Daood et al., 2015), C18 reversed phase column HPLC (Sanatombi and Sharma, 2008), reverse phase High performance liquid chromatography/Photodiode array detection (RP-HPLC/ PAD) (Gahungu et al., 2011), and HPLC-UV spectrometry-Diode Array Detector (Giuffrida et al., 2013). Other methods focus on ultra-fast liquid chromatography (UFLC) (Usman et al., 2014).

2.5 CHILI PRESERVATION BY DRYING

The outstanding nutritive and culinary value of chili peppers endow them high demand on the market all year long. Nevertheless, as any other food produce chilies are noticeably perishable; two weeks of storage at ambient temperature are enough to make pepper fruits unmarketable (Samira et al., 2013). Chili peppers are substantially sensitive to temperature and humidity conditions and prone to postharvest diseases or infections caused by *Botrytis*, *Alternaria*, and soft rots of fungal and bacterial origin (Cantwell, 2009). Thus, suitable postharvest management and preservation methods are needed to ensure extended shelf life and high quality of peppers.

Drying is the oldest and most common method used for food preservation. Sufficient reduction of water activity in the food impedes the growth of harmful microorganisms like bacteria and yeasts (Tasirin et al., 2007). It guarantees long shelf-life of products, while maintaining expected quality attributes of the products. Additionally, decreased weight and volume result in reduced transport and storage costs, plus environmental advantages (Lewicki, 2006).

2.5.1 Drying principles

Drying is defined as the application of heat under controlled conditions to remove most of the water contained in food products by evaporation, or sublimation in the case of freeze drying (Fellows, 2009). A Product undergoing a drying process decreases in weight as moisture is evaporated from it, until reaching a desired final moisture content. The rate at which food dries may vary (see Figure 5) depending on factors related to the processing conditions, nature of the food, and the drier design.

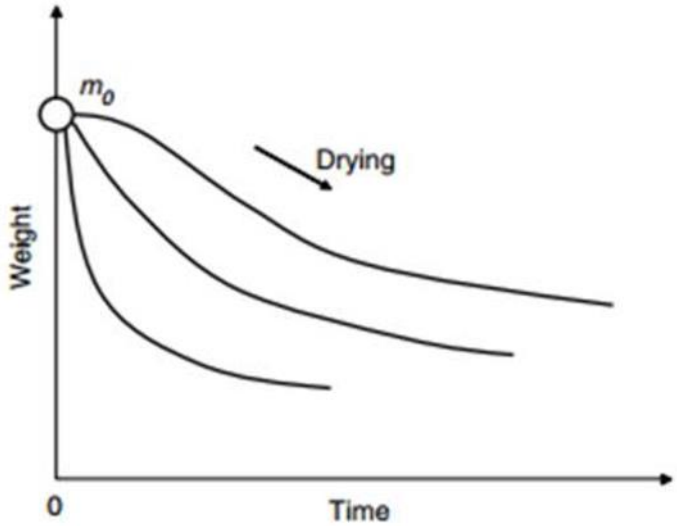


Figure 5 Different trends of weight loss as drying proceeds (Chen and Mujumdar, 2008).

Drying processes can be classified in three different categories: air and contact drying, in which heat is transferred to food through the heated air or surfaces and vapour is removed by air; vacuum drying which takes advantage of the fact that evaporation of water occurs more readily at lower pressures than at higher ones; and freeze drying in which the vapor of water is sublimed off frozen food.

Convection (hot air) drying is among the most used methods for food dehydration. It is a dual process consisting in a simultaneous heat and mass transfer. Moisture is transferred to the surface of the food product by diffusion and then pulled away into the air flow by convection (see Figure 6*)*. At the same time, heat is transferred by convection to air-food product interface and transferred to the interior of the fruits by conduction (Zogzas and Maroulis, 1996).

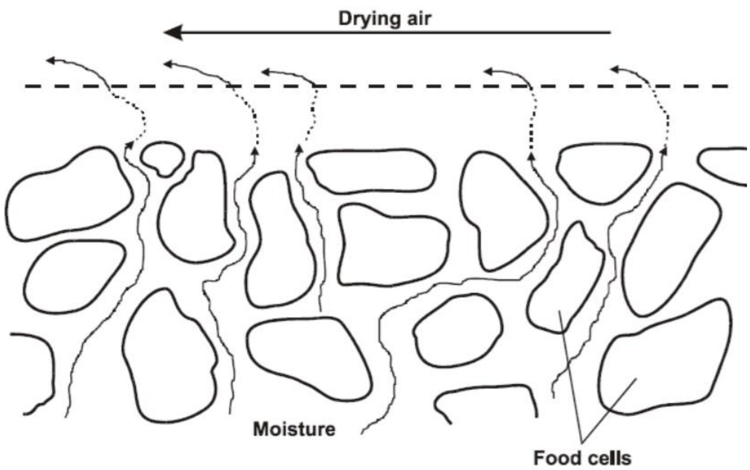


Figure 6 Air taking away moisture from food tissues(Brenndorfer et al., 1985).

The capacity of air to remove moisture from foods is controlled by three inter-related factors: the amount of water vapor already carried by the air, the air temperature, and the amount of air that passes over the food. In order to achieve a successful drying, low RH, a moderately high dry-bulb temperature and a high air velocity are recommended (Fellows, 2009).

2.5.2 Quality changes during drying

Applying heat to food materials does not only remove the moisture but also causes physical, chemical and biological changes that affect the product quality. These alterations include oxidation, shrinkage, loss in colour and texture as well as the loss in nutritional and functional properties of the resultant product (Cankaya et al., 2017). Some of the most common phenomena affecting the quality of food during drying are listed below.

Browning

Browning or discoloration is caused by physical (thermal breakdown) or chemical (enzymatic and non-enzymatic) reactions. Both discoloration and drying rate increase with higher drying temperatures therefore an optimum must be reached to reduce the drying time and obtain an acceptable colour of the final product (Brenndorfer et al., 1985). Temperatures lower than 100 °C are recommended to keep the colour and brightness properties of chilies undergoing drying treatments (Rochín-Wong et al., 2013). Nevertheless, chilies subjected to hot air drying, between 50 and 70 °C, have shown up to 27 % colour decrease (Mihindukulasuriya and Jayasuriya, 2015). Final moisture is also an important factor contributing to the stability of ascorbic acid and pigment in dried chilies; moisture contents over 8 % are recommended to prevent colour loss (Toontom et al., 2012).

Migration of soluble constituents

The evaporation of water at elevated temperatures induces the migration of soluble constituents and their succeeding concentration in the surface of food material. It also causes alterations in pH, redox potential and solubility of biopolymers. These soluble substances (reagents and catalysts), added to the heightened availability of oxygen within the food tissues, promote chemical and enzymatic reactions in the last stages of drying (Lewicki, 2006). Among them, the enzymatic activity of peroxidase is involved with capsaicinoids degradation (Contreras-Padilla and Yahia, 1998).

Case hardening

Case hardening can be regarded as one example of the effects of the migration of soluble constituents to surface layers during drying. This phenomenon hinders the drying process of whole fruits and vegetables as the surface of food materials become dry and impermeable to additional flow of moisture, and therefore considerable moisture remains trapped within. Case hardening can be avoided by drying fruits in slices or pieces; the increased surface facilitates the migration of moisture through the material in the later stages of drying (Brenndorfer et al., 1985).

Loss of volatile constituents

The moisture dissipating in the form of water vapour, during drying, carries with it traces of every other volatile constituent present in the food product. The concentration of a volatile substance in the water vapour depends on its vapour pressure, its solubility, and those of the other constituents, in water (Brenndorfer et al., 1985). Volatile constituents are of great importance in spice products since their loss affects remarkably their flavour and aroma properties. Some studies have shown a significant impact on the content of carotenoids and capsaicinoids in chili peppers subjected to different heat treatment (Topuz and Ozdemir, 2004; Yaldiz et al., 2010; Mihindukulasuriya and Jayasuriya, 2015).

Regarding the content of capsaicinoids, Yaldiz et al. (2010) analysed six cultivars of chilli peppers under 3 different drying methods (oven drying, sun drying and solar drying) at different temperatures; results showed significant variations regarding the method employed, and negative correlation between capsaicin content and temperature. Similarly, Mihindukulasuriya and Jayasuriy (2015) showed that an increase in drying temperature from 50 °C to 70 °C could decrease capsaicinoid content by 25 %. Notwithstanding, Victoria-Campos et al. (2015) reported a 1.6-fold increase in the content of dihydrocapsaicin of boiled peppers while capsaicin presented no changes, suggesting individual capsaicinoids may behave differently under thermal influence. Moreover, Toontom et al. (2012) found significantly greater capsaicinoid contents on oven-dried, freeze-dried and sun-dried *C. annuum* peppers compared to fresh fruits.

3 OBJECTIVES

The main objective of this research was to investigate the influence of drying on the final content of capsaicinoids in selected Capsicum species.

Specific objectives were to measure the content of capsaicin and dihydrocapsaicin in the selected varieties of chili peppers by means of GC-MS. Furthermore, to determine the effect of different drying pre-treatments and drying temperatures on the final content of those capsaicinoids in the selected chili peppers.

4 MATERIALS AND METHODS

4.1 PLANT MATERIAL

Eleven different Capsicum varieties were randomly selected from the botanical garden of the Faculty of Tropical AgriSciences, Czech University of Life Sciences (CULS) Prague (see Figure 7). Four genotypes of *Capsicum annuum* L. peppers identified as Hot Jalapeño (HJA), Cheyene Lutea (CLU), Black Plum (BPL), and Fantasia (FAN); four genotypes of *Capsicum chinense* Jacq. peppers identified as Timoito Pegueño (TPE), Trinidad Congo Yellow (TCY), Rubo Roso (RRO), and Habareno Bonda (HBO); one genotype of *Capsicum baccatum* L. identified as Christmas Bells (CBE); and two genotypes of *Capsicum frutescens* L., Twilight (TWI), and one not specified (NA).

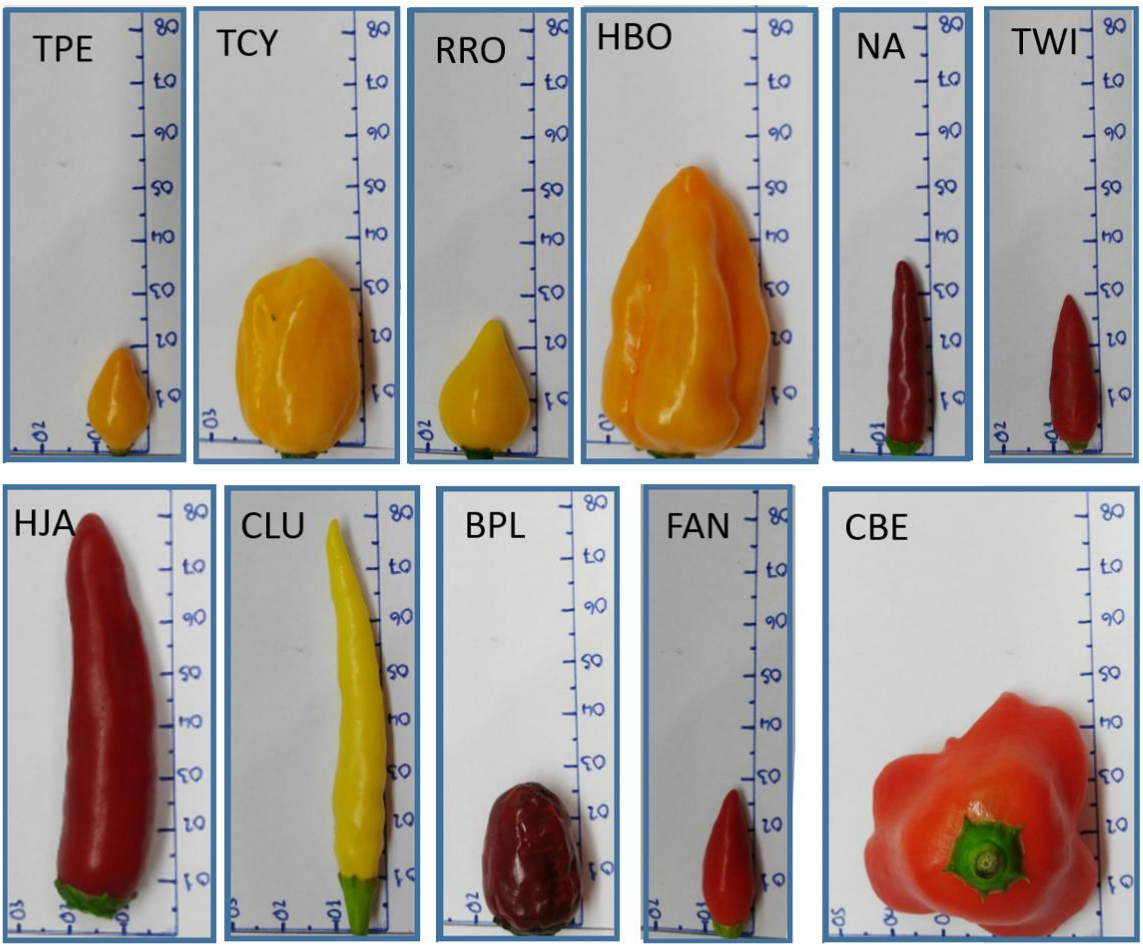


Figure 7 Fully ripen chili peppers harvested for the study. Photos taken in the laboratoy.

Fully ripen fruits were harvested and then separated into two groups: peppers to be analysed as fresh samples, packed and frozen at -18 °C and then thawed 24 h in the refrigerator for extraction; and peppers to be oven-dried, stored in the refrigerator at 4 °C until treated. Most of the selected chili peppers have not been previously analysed; the determination of capsaicinoid content in these varieties is crucial information for their future analysis.

4.2 REAGENTS

Analytical standards of capsaicin (≥99.0 % (HPLC)12084-10MG-F) and dihydrocapsaicin (≥ 97.0 % (HPLC) 03813-5MG), HPLC and GC suitable, were used for detection and quantitation. Standard capsaicinoids were purchased from Sigma-Aldrich (Prague, Czech Republic).

Solvents, Methanol (Methylalkohol p.a. 21210-99.8 %) purchased from Ing. Petr Švec - PENTA s.r.o. (Prague, Czech Republic); and n-Hexane (98 %) for gas chromatography MS SupraSolv purchased from Merk Millipore (Prague, Czech Republic); were used for capsaicinoid extraction and measurement, respectively.

4.3 DRYING EXPERIMENT

Chili peppers were prepared in three different ways: whole, cut in half, and mashed with mortar and pestle. Samples (2 to 5 g) were oven-dried separately at 40 and 60 °C for 3 to 5 days (depending on the fruit size); final moisture content ranged between 10 and 15%, complying with proper storage conditions(WFLO, 2010). The climate box POLEKO KKP 115 was used for this purpose. Dried samples were stored in plastic bags at ambient temperature until processed.

4.4 EXTRACTION

The capsaicinoid extraction was carried out as described by previous studies (Collins et al., 1995; Topuz et al., 2011), with a few modifications. Both fresh and dried samples were ground, subsequently blended with methanol and poured in 4ml glass vials;

solvent volume-to-pepper weight ratio was 5 ml per gram of fresh pepper weight. Vials were capped and placed in a water bath at 60°C for 4 hours. Suspensions were manually swirled every 30 minutes throughout the extraction process; later removed from the water bath and cooled to room temperature.

One millilitre of supernatant was extracted and filtered using a Hamilton SYR 500 µl 750N (ga22/51mm/pts2) glass syringe decontaminated with methanol every time before filtering, on to a 2-ml sample glass vial, then capped and stored at 4 °C until analysed. All samples were measured within 2 days of extraction.

4.5 GC-MS ANALYSIS

Gas chromatography-mass spectrometry (GC-MS) was used for the separation, identification and quantitation of capsaicin an dihydrocapsaicin in both fresh and dried peppers. Triplicate injections were effectuated on each analytical sample.

Gas chromatography is a widely applied technique in organic and inorganic chemistry with the fundamental role of separating the individual volatile compounds from complex mixtures in a short time. Nonetheless, the ability of gas chromatography to establish the nature and chemical structure of separated compounds is ambiguous and reduced (Stashenko and Martínez, 2014). To compensate this disadvantage, mass spectrometers are used as detectors; GC-MS provide the unique opportunity to obtain the mass spectrum for each chromatographic peak and the shoulders and baselines in the chromatogram (Eiceman et al, 2000)

Gas chromatography-mass spectrometry was carried out utilising a nonpolar capillary column Agilent DB5MS (5 % - phenyl methyl siloxane, 30.0 cm × 0.25 mm × 0.25 µm) on an Agilent 7890B GC System – 5977A MSD, equipped with auto sampler. The gas chromatographic conditions were as follow: Carrier gas, Helium (99,99 %) flowing at 1ml/min; initial furnace temperature was set at 60°C for 2 minutes then programmed to 280 °C at a rate of 15 °C/min; the injector temperature was 250 °C, in split less mode; injected volume:1 µl. The MS ionization potential was 70 eV, and interface temperature at 270 °C. The mass spectrometer was operated in the full scan mode from 32 to 600 m/z. The software used for acquisition and quantitative analysis was Agilent MassHunter Workstation (version B.07.01/Build 7.1.524.0).

Capsaicinoid standards were used for instrument calibration. Separate standard solutions for capsaicin and dihydrocapsaicin, of 50, 10 and 5 ppm were prepared in 100 % methanol by dilution of a 1000 ppm stock solution. Regression equations with their corresponding correlation coefficients, and the limits of detection (LOD) and quantitation (LOQ) were determined according to Shirvastava and Gupta (2011). All calculations were made with MATLAB R2015a software.

4.6 SCOVILLE HEAT UNITS DETERMINATION

The relation between capsaicinoids content and Scoville heat units has been previously described by means of comparative data for pungency determined organoleptically and by GLC on samples ranging from 50,000- 2,000,000 SHU (Todd et al., 1977).

Table 2 Threshold pungencies of capsaicinoids (millions).

Capsaicin	16.1 ± 0.6
Dihiydrocapsaicin	16.1 ± 0.6
Nordihydrocapsaicin	9.3 ± 0.4
Homocapsaicin	6.9 ± 0.5
Homodihydrocapsaicin	8.1 ± 0.7
Vanillyl pelargonamide	9.2 ± 0.5

Pungency determination for the two main capsaicinoids was accomplished by multiplying their individual capsaicinoid contents and their corresponding pungency threshold value(Gahungu et al., 2011).

$$SHU = [(\%Capsaicin \times 16.1) + (\%\ Dihydrocapsaicin \times 16.1)] \times 10{,}000$$

4.7 STATISTICAL ANALYSIS

Samples were analysed in triplicates and data was reported in mean ± standard deviation. One way analyses of variance (ANOVA) with post hoc Tukey Honest Significant Difference test was used to compare the significant differences beteween the fresh samples and each of the pre-treatments at a confidence level of $\alpha=0.95$ ($p<0.05$). All statistical analyses of the experimental data were preformed using MATLAB R2015a software.

5 RESULTS

Capsaicin and dihydrocapsaicin were successfully identified and quantified in all the *Capsicum* varieties. Chromatograms and spectra for capsaicin and dihydrocapsaicin standards are shown in Figure 8. Retention times were 18.471 min and 18.637 min, respectively for capsaicin and dihydrocapsaicin. Calibration curves were defined for the two standards; regression results, limits of detection, and limits of quantitation are shown in Table 3. Correlation coefficients r^2 obtained were both > 0.999.

Table 3 *Calibration results for capsaicin and dihydrocapsaicin standards. ^{A}LOD: Limits of detection. ^{B}LOQ: Limits of quantitation.*

	Capsaicin	Dihidrocapsaicin
Equantion	$y = 3.2160 \cdot 10^7 x - 1.4277 \cdot 10^5$	$y = 2.8712 \cdot 10^7 x - 1.6828 \cdot 10^5$
r^2	0.9999	0.9992
LOD^A (mg/ml)	0.0011	0.0035
LOQ^B(mg/ml)	0.0027	0.0092

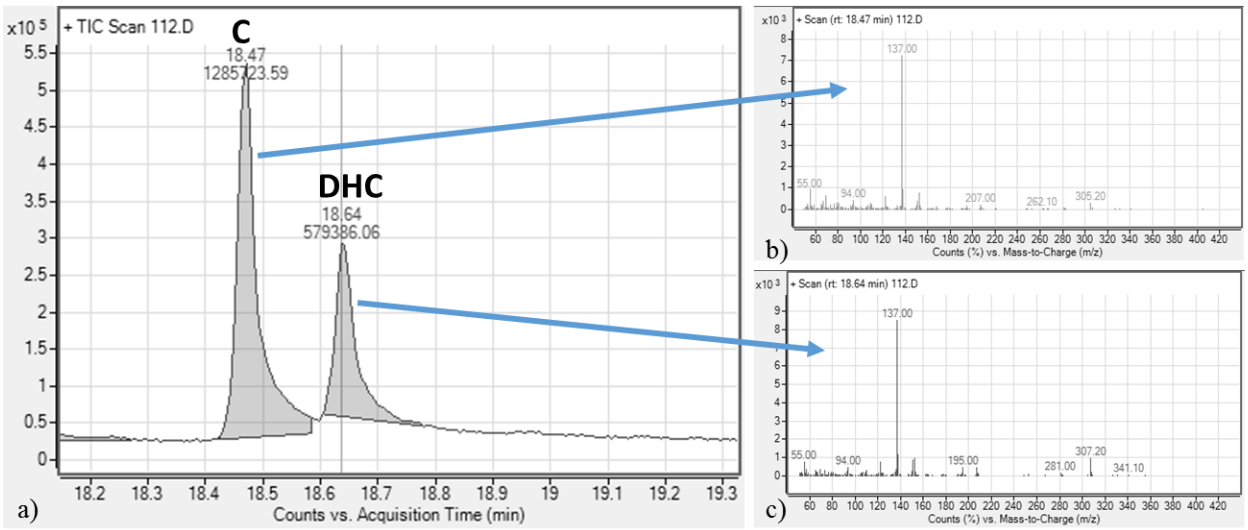


Figure 8 a) Chromatogram for capsaicin (C) and dihydrocapsaicin (DHC) standards. b) Capsaicin mass spectra, c) Dihydrocapsaicin mass spectra.

5.1 CAPSAICINOID CONTENT IN FRESH PEPPERS

Capsaicin and dihydrocapsaicin contents were determined for the eleven chili pepper cultivars. Individual capsaicinoid contents are presented in µg/g (dry basis). Total pungency of chili peppers, obtained by adding capsaicin and dihydrocapsaicin contents, are shown in Scoville heat units (SHU).

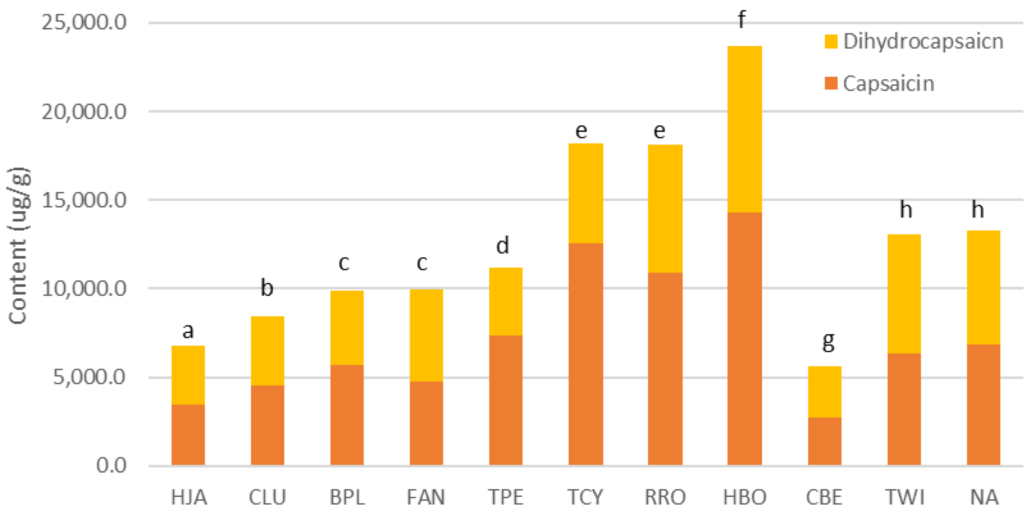
Results of capsaicinoid content for fresh peppers varied significantly ($p<0.05$) among *Capsicum* cultivars. Only pairs Black Plum-Fantasia, Trinidad Congo Yellow-Rubo Roso, and Twilight-NA, can be regarded as having the same level of pungency. Values for capsaicin ranged from 2759.8 to 14281.7 with standard deviations up to 3.5 %. *C. chinense* Jacq. peppers had the highests amounts of capsaicin, followed by *C. frutescens* L., *C. annuum* L., and *C. baccatum* L. fruits. Habanero Bonda obtained the highest capsaicin content, around 4 times the content in Hot Jalapeño. Dihydrocapsaicin contents ranged from and 2887.9 to 9422.6 with standard deviations up to 1.6 %. Once again, concentration was highest in Habanero Bonda and lowest in Christmas Bells, though results did not follow the same pattern of capsaicin in the rest of the peppers.

Table 4 Capsaicinoid content for the 11 varieties of fresh chili peppers.

	Capsaicin		Dihydrocapsaicn			Total Capsaicinoids
	µg/g	± SD	µg/g	± SD	C : DHC	SHU
Hot Jalapeño	3,459.5 a	± 59.1	3,321.6 a	± 34.1	1.04 : 1	109,176 a
Cheyene Lutea	4,524.0 b	± 135.5	3,922.8 b	± 45.2	1.15 : 1	135,994 b
Black Plum	5,696.9 c	± 45.2	4,218.5 c	± 34.1	1.35 : 1	159,638 c
Fantasia	4,790.1 b	± 59.1	5,145.0 d	± 29.6	0.93 : 1	159,955 c
Timoito pequeño	7,352.8 d	± 239.0	3,804.5 b	± 17.1	1.93 : 1	179,632 d
Trinidad Congo Yellow	12,547.0 e	± 95.1	5,657.5 e	± 90.3	2.22 : 1	293,093 e
Rubo Roso	10,910.9 f	± 0.0	7,214.8 f	± 29.6	1.51 : 1	291,823 e
Habanero Bonda	14,281.7 g	± 88.7	9,422.6 g	± 103.8	1.52 : 1	381,639 f
Christmas Bells	2,759.8 h	± 17.1	2,887.9 h	± 17.1	0.96 : 1	90,927 g
Twilight	6,327.7 i	± 78.2	6,741.7 i	± 59.1	0.94 : 1	210,417 h
NA	6,860.0 j	± 29.6	6,396.7 j	± 61.6	1.07 : 1	213,432 h

Note: Each value is an average of three samples + standard error of the mean. The means followed by different letters in the same column are significantly different ($p < 0.05$) by Tukey's test. C:DHC shows the ratio between capsaicin and dihydrcapsaicin contents.

Relative proportion between capsaicin and dihydrocapsaicin may vary from 1:1 to 2:1 (Orellana-Escobedo et al., 2013). An average factor of 1.3 was calculated for the capsaicin to dihydrocapsaicin ratio. Capsaicin content was 1.5-fold to 2.2-fold compared to dihydrocapsaicin in *C. chinense* Jacq. fruits, and 1.35-fold in Black Plum. Meanwhile, all the other chili peppers had a ratio around 1:1. Although capsaicin is usually the most abundant capsaicinoid, dihydrocapsaicin was more abundant in the chili peppers: Fantasia, Twilight and Christmas Bells. González-Zamora et al. (2013) have also identified higher content of dihydrocapsaicin in 7 accessions of *C. annuum* which presented a presented a 1:2 ratio, nevertheless the study was done in unripen peppers.



Note: bars with different letters are significantly different ($p < 0.05$) by Tukey's test.

Figure 9 Capsaicin and Dihydrocapsaicin contents in fresh peppers.

Regarding the total content of capsaicinoids, as expected Habanero Bonda registered the highest values (381,639 SHU), followed by Trinidad Congo Yellow (293,093 SHU) and Rubo Roso (291,823 SHU), which also belong to the *C. chinense* genus. Despite no previous study has characterised pungency in these specific accessions, results are similar to those reported for other highly pungent peppers of the *C. chinense* genus (Giuffrida et al., 2013; Orellana-Escobedo et al., 2013). Kurian and Starks (2002) analysed capsaicinoids on fresh Habanero Orange obtaining similar contents for capsaicin (8,820 µg/g) and dihydrocapsaicin (3,940 µg/g). Other studies on whole fresh Habanero type chilies support these results (Chinn et al., 2011). In contrast, other authors have reported much lower capsaicinoid contents in Habanero Orange and White, perhaps because samples were frozen before analysed (Cisneros-Pineda et al., 2007).

The chili with the lowest values of pungency was Christmas Bells (90,927 SHU), belonging to the *C. baccatum* L. This results agree with Kollmannsberger et al. (2011) who reported *C. chinense* as the species having many of the most pungent *Capsicum* genotypes and *C. baccatum* as the species having some of the least pungent fruits. Anyhow, all the varieties studied can be regarded as medium or highly pungent.

The second least pungent pepper was Hot Jalapeño (109,175.8 SHU). Results were much higher than previous results, 9,400 SHU and 36,943 SHU, found for the Jalapeño peppers (Orellana-Escobedo et al., 2013; Victoria-Campos et al., 2015). Nonetheless, even higher results (303,602 SHU) have been reported for the Jalapeño cultivars (González-Zamora et al., 2013).

5.2 INFLUENCE OF DRYING METHODS ON CAPSAICINOIDS

The effect of drying on the capsaicinoid content was significant for most of the chili peppers (see Table 7*).* Capsaicin and dihydrocapsaicin behaved similarly, but capsaicin to dihydrocapsaicin proportion factor changed considerably (0.94-1.23) in each *Capsicum* cultivar. Capsaicin was highly sensitive to the drying process, changing 14 % in average, but increasing up to 93 % and decreasing as much as 67 % compared to the contents in fresh fruits of specific chili peppers. Capsaicin contents change significantly for all peppers but Hot Jalapeño, Black Plum, Cheyene Lutea and Timoito Pequeño, compared to fresh peppers.

Dihydrocapsaicin was less sensitive to the drying process, decreased from 5 to 22 % in most peppers, and did not register any significant changes when evaluated separately. Nevertheless, when dihydrocapsaicin is evaluated together with capsaicin, significant changes were found for Cheyene Lutea; meaning that only Jalapeño, Black Plum, and Timoito Pequeño did not present significant variations in terms of total capsaicinod content. Capsaicin and total capsaicinoid contents also varied differently regarding the conditions of the drying process and the *Capsicum* genus. Among the different drying processes, the highest contents of capsaicin and total pungency were found in Trinidad Congo Yellow, 15,760.2 µg/g and 344,824.1 SHU, respectively.

Table 5 Capsaicin contents (µg/g) by drying process for the 11 chili peppers.

Varieties		Drying Process					
	Fresh Peppers	Whole peppers		Sliced by half		Mashed	
		40 °C	60 °C	40 °C	60 °C	40 °C	60 °C
Hot Jalapeño	3,459.5 ab ± 59.1	2,749.9 a ± 0.0	2,779.5 b ± 0.0	2,789.3 a ± 17.1	2,749.9 a ± 0.0	2,848.5 a ± 17.1	2,740.0 a ± 17.1
Cheyene Lutea	4,524.0 a ± 135.5	4,317.0 a ± 128.9	4,257.9 a ± 135.5	4,287.5 a ± 51.2	4,021.4 a ± 59.1	3,341.3 a ± 29.6	3,686.2 a ± 45.2
Black Plum	5,696.9 ab ± 45.2	4,110.1 ab ± 51.2	4,543.7 a ± 74.4	3,607.4 ab ± 59.1	4,149.5 ab ± 17.1	2,917.5 b ± 34.1	3,311.7 ab ± 29.6
Fantasia	4,790.1 ad ± 59.1	7,037.4 b ± 59.1	6,278.4 ad ± 290.2	9,225.5 c ± 252.6	5,371.7 d ± 95.1	2,740.0 e ± 17.1	4,031.2 d ± 17.1
Timoito pequeño	7,352.8 a ± 239.0	4,583.2 a ± 59.1	5,135.1 a ± 61.6	5,420.9 a ± 111.9	4,632.4 a ± 45.2	3,006.2 a ± 74.4	4,878.8 a ± 88.7
Trinidad Congo Yellow	12,547.0 a ± 95.1	14,587.3 b ± 168.1	6,623.4 b ± 59.1	15,760.2 c ± 29.6	12,142.9 a ± 332.3	14,045.2 ac ± 88.7	4,090.3 b ± 74.4
Rubo Roso	10,910.9 a ± 0.0	4,573.3 b ± 45.2	4,366.3 b ± 90.3	8,308.8 ac ± 257.8	4,267.8 b ± 90.3	4,336.8 b ± 268.3	6,051.7 bc ± 45.2
Habanero Bonda	14,281.7 a ± 88.7	8,722.8 b ± 78.2	6,978.2 c ± 118.3	5,154.8 c ± 111.9	7,707.6 bd ± 119.5	9,136.8 b ± 184.7	7,293.6 d ± 74.4
Christmas Bells	2,759.8 a ± 17.1	2,996.3 a ± 61.6	3,242.7 b ± 17.1	3,282.1 ab ± 29.6	3,114.6 ab ± 34.1	2,759.8 a ± 17.1	2,759.8 a ± 17.1
Twilight	6,327.7 a ± 78.2	9,205.7 a ± 74.4	6,239.0 a ± 256.1	12,172.5 b ± 357.7	6,672.7 a ± 111.9	6,860.0 a ± 300.1	6,012.3 a ± 17.1
NA	6,860.0 a ± 29.6	12,133.1 b ± 68.3	7,609.0 c ± 45.2	5,647.6 d ± 59.1	8,663.6 e ± 29.6	3,755.2 c ± 29.6	4,366.3 c ± 95.1

Note: Each value is an average of three samples ± standard error of the mean. The means followed by different letters in the same row are significantly different ($p < 0.05$) by Tukey's test.

Table 6 Dihydrocapsaicin contents (µg/g) by drying process for the 11 chili peppers.

Varieties		Drying Process					
	Fresh Peppers	Whole peppers		Sliced by half		Mashed	
		40 °C	60 °C	40 °C	60 °C	40 °C	60 °C
Hot Jalapeño	3,321.6 a ± 34.1	2,878.0 a ± 17.1	2,937.2 a ± 17.1	2,917.5 a ± 17.1	2,887.9 a ± 17.1	3,045.6 a ± 0.0	3,223.0 a ± 153.6
Cheyene Lutea	3,922.8 a ± 45.2	3,903.1 a ± 147.8	3,814.4 a ± 51.2	3,696.1 a ± 51.2	3,706.0 a ± 85.4	3,311.7 a ± 29.6	3,292.0 a ± 45.2
Black Plum	4,218.5 a ± 34.1	3,459.5 a ± 29.6	3,577.8 a ± 29.6	3,351.1 a ± 34.1	3,597.5 a ± 45.2	3,055.4 a ± 17.1	3,262.4 a ± 17.1
Fantasia	5,145.0 a ± 29.6	6,662.8 a ± 170.7	5,903.9 a ± 74.4	8,062.4 a ± 621.9	5,351.9 a ± 29.6	2,897.7 a ± 0.0	4,228.3 a ± 78.2
Timoito pequeño	3,804.5 a ± 17.1	3,223.0 a ± 51.2	3,282.1 a ± 0.0	3,282.1 a ± 0.0	3,183.6 a ± 45.2	2,878.0 a ± 17.1	3,183.6 a ± 45.2
Trinidad Congo Yellow	5,657.5 a ± 90.3	5,943.3 a ± 29.6	3,794.7 a ± 74.4	5,657.5 a ± 190.1	4,770.4 a ± 34.1	6,928.9 a ± 68.3	3,321.6 a ± 17.1
Rubo Roso	7,214.8 a ± 29.6	3,686.2 a ± 393.8	3,479.3 a ± 17.1	5,549.1 a ± 332.3	3,301.8 a ± 61.6	3,262.4 a ± 17.1	3,587.7 a ± 17.1
Habanero Bonda	9,422.6 a ± 103.8	6,978.2 a ± 262.8	5,105.5 a ± 74.4	4,248.0 a ± 17.1	6,485.4 a ± 90.3	7,037.4 a ± 333.2	5,223.8 a ± 68.3
Christmas Bells	2,887.9 a ± 17.1	3,154.0 a ± 45.2	3,223.0 a ± 29.6	3,390.6 a ± 45.2	3,193.4 a ± 51.2	2,887.9 a ± 17.1	2,947.0 a ± 74.4
Twilight	6,741.7 a ± 59.1	7,185.2 a ± 102.4	5,509.6 a ± 239.0	8,949.5 a ± 385.9	6,347.4 a ± 17.1	5,854.6 a ± 406.5	4,435.3 a ± 118.3
NA	6,396.7 a ± 61.6	6,860.0 a ± 51.2	4,740.9 a ± 45.2	4,395.9 a ± 34.1	6,120.7 a ± 29.6	3,449.7 a ± 17.1	4,435.3 a ± 78.2

Note: Each value is an average of three samples ± standard error of the mean. The means followed by different letters in the same row are significantly different ($p < 0.05$) by Tukey's test.

Table 7 Estimated pungency values (SHU) for the 11 varieties of chili peppers by drying process.

Varieties	Fresh Peppers	Drying Parameters: Whole peppers		Sliced by half		Mashed	
		40 °C	60 °C	40 °C	60 °C	40 °C	60 °C
Hot Jalapeño	109,175.8 a	90,609.6 a	92,037.7 a	91,879.0 a	90,768.2 a	94,894.1 a	96,004.9 a
Cheyene Lutea	135,993.7 a	132,343.9 ab	129,963.6 b	128,535.4 ab	124,409.6 a	107,112.9 b	112,349.5 a
Black Plum	159,637.8 a	121,870.6 a	130,757.0 a	112,032.1 a	124,727.0 a	96,163.6 a	105,843.4 a
Fantasia	159,955.2 ab	220,573.2 c	196,135.6 bc	278,334.8 c	172,650.1 ab	90,768.2 a	132,978.6 a
Timoito pequeño	179,632.3 a	125,679.1 a	135,517.6 a	140,119.5 a	125,837.8 a	94,735.4 a	129,804.9 a
Trinidad Congo Yellow	293,092.6 abc	330,542.4 b	167,730.8 d	344,824.1 b	272,304.7 c	337,683.2 abc	119,331.7 d
Rubo Roso	291,823.1 a	132,978.6 b	126,313.8 bcd	223,112.1 ac	121,870.6 bd	122,346.7 bcd	155,194.6 bcd
Habanero Bonda	381,639.2 a	252,786.4 b	194,548.7 b	151,386.2 b	228,507.5 b	260,403.3 b	201,530.9 b
Christmas Bells	90,926.9 a	99,019.9 ab	104,097.8 b	107,430.2 ab	101,558.9 ab	90,926.9 ab	91,879.0 ab
Twilight	210,417.3 ab	263,894.4 cd	189,153.4 ab	340,063.5 d	209,623.9 ab	204,704.6 ac	168,206.9 b
NA	213,432.3 ab	305,787.4 c	198,833.2 bc	161,700.8 c	238,028.6 ab	115,999.3 a	141,706.4 a

Note: Each value is an average of three samples. The means followed by different letters in the same row are significantly different ($p < 0.05$) by Tukey's test.

5.2.1 Whole fruits

Significant changes were found for chili peppers dried as whole fruits. Variations on the total content of capsaicinoids were dependent on the *Capsicum* cultivar (see Figure 10). Significant increase in the total content of capsaicinoids was found in Fantasia (38 %), Twilight (25 %), and NA (43 %) at 40 °C. Capsaicinoid content in Trinidad Congo Yellow also increased at 40 °C but not in a significant amount. Conversely, Habanero Bonda and Rubo Roso decreased significantly at 40 °C, 39 % and 58 % respectively. At 60 °C, capsaicinoids content decreased for almost all peppers, but values were only statistically different for Cheyene Lutea, Trinidad Congo Yellow, Rubo Roso and Habanero Bonda, compared to fresh samples. Dried Twilight and Trinidad Congo Yellow presented significant changes between the capsaicinoids contents at 40 °C and 60 °C.

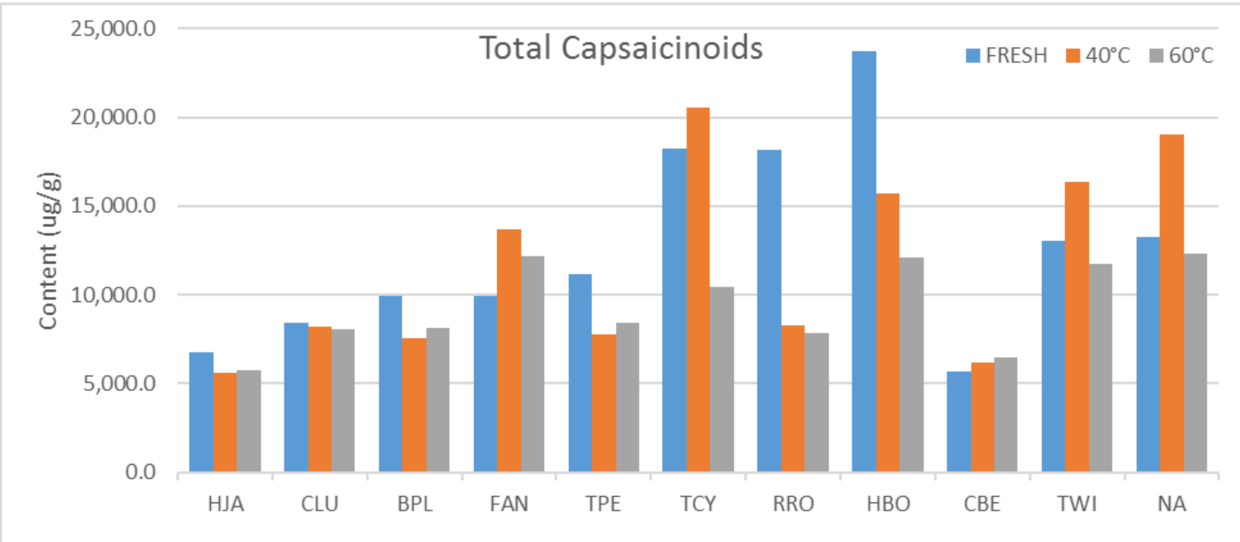


Figure 10 Influence of Whole fruit drying in total capsaicinoid content (μg/g).

5.2.2 Chilies cut in Half piece

Four out of the eleven chili peppers, cut by half and dried, presented significant changes compared to fresh samples: Fantasia, Rubo Roso, Habanero Bonda, and Twilight. Capsaicinoid content for both Fantasia and Twilight almost doubled at 40 °C (see Figure 11). Trinidad Congo Yellow increased once more, though not significantly.

Meanwhile, content in Habanero Bonda decreased 64 % at the same temperature. At 60 °C, significant reduction in pungency was shown in Habanero Bonda and Rubo Roso, by 46 % and 61 %, respectively. Within the half-cut treatment, significant changes due to temperature were present in the following chilies: Trinidad Congo Yellow, Twilight, Fantasia, and Rubo Roso; pungency values decreased as temperature augmented.

Concerning significant changes between whole-fruit and half-cut treatments, pungency raised in Rubo Roso and lessend in NA when treated by half-cut at 40 °C. At 60 °C, capsaicinoids content increased in Trinidad Congo Yellow and decreased in Cheyene Lutea.

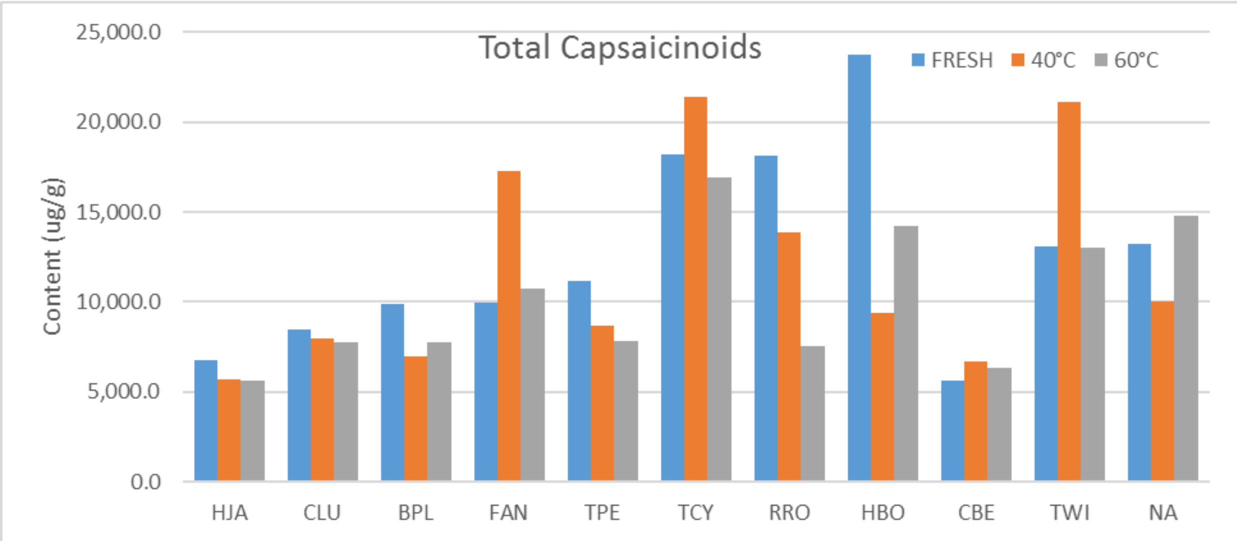


Figure 11 Influence of Half slice drying pre-treatment in total capsaicinoid content (µg/g).

5.2.3 Mashed Chilies

Mashed pre-treatment influenced the total capsaicinoids content in four of the studied chili peppers. Habanero Bonda and Rubo Roso were the only varieties that exhibited changes ($p<0.05$) at 40 °C decreasing 32 % and 58 %, respectively (see Figure 12). At this temperature, the capsaicinoid content decreased as well on *Capsicum* varieties that had exhibited higher values for the previous pre-treatments (FAN and

TWI). However, content in Trinidad Congo Yellow remained greater. At 60 °C, contents in all *Capsicum* varieties but Christmas Bells decreased. Habanero Bonda and Rubo Roso, NA and Trinidad Congo Yellow showed significant reductions in capsaicinoid content by 47 %, 47 %, 34 % and 59 %, respectively.

Significant differences within the same treatment were found in 3 chilies. The capsaicinoids content for both Trinidad Congo Yellow and Habanero Bonda diminished with the rise of temperature. On the other hand, the value for Fantasia increased at 60 °C. In regards to the significant variations among pre-treatments, mashed Fantasia and NA lowered in relation to whole-pepper and half-cut pre-treatments, at 40 °C. Mash pre-treated Cheyene Lutea, Fantasia and NA decreased regarding whole peppers dried at 60 °C. At the same temperature, Trinidad Congo Yellow and NA pungency values also decreased in relation to half-cut peppers.

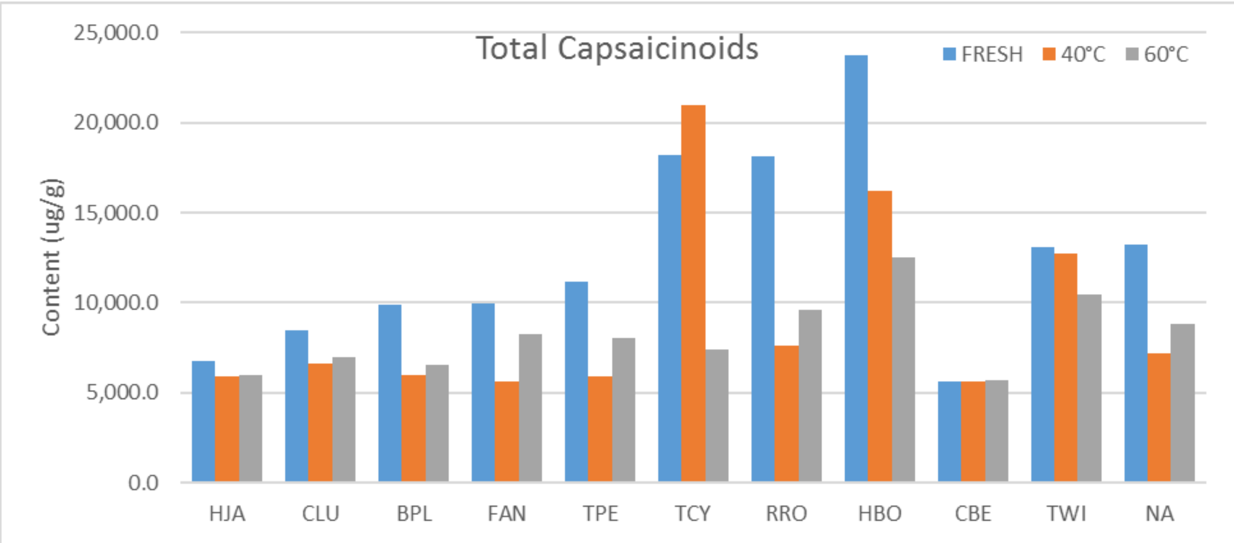


Figure 12 Influence of mashed drying pre-treatment in total capsaicinoid content (µg/g).

6 DISCUSSION

The effect of pre-treatment and drying temperature on the final content of capsaicinoids was investigated in this study. ANOVA analyses indicated that none of the pre-treatments alone nor the temperatures had statistical significant influence on the content of total capsaicinoids. Results depended mainly on the chili pepper variety, and changed importantly even among cultivars within the same *Capsicum* genus. González-Zamora et al. (2013) reported that changes in capsaicinoids, due to drying, decreased as much as 61.5 % and increased up to 21 %, among different cultivars. While capsaicinoids in some chilies (Hot Jalapeño, Black Plum, Timoito Pequeño) remained unchanged, Habanero Bonda presented a significant decrease on capsaicinoids for all the different drying methods.

As a general trend, total capsaicinoids on most chili peppers decreased when subjected to drying at 60 °C, except NA when prepared in half-piece; yet not all changes were significant. The negative impact of drying temperature on capsaicinoids content has been evidenced by other authors (Yaldiz et al., 2010; Topuz et al., 2011). Rochín-Wong et al. (2013) explained that lower capsaicinoids amounts may be caused by the catalytic activity of oxidising enzymes on capsaicinoids, especially peroxidase, which breaks down alkyl groups of capsaicinoids into vanillin and other phenols.

Chili peppers prepared as whole fruits and as mashed samples, dried at 60 °C, showed greater affections on capsaicinoids contents than those pre-treated in half-piece. Topuz et al. (2011) reported higher capsaicinoids losses in puree-prepared Jalapeño, oven-dried at 60 °C, compared to freeze drying and natural convection drying because of higher temperature. Puree or mashed preparations may be more sensitive to temperature changes as larger surfaces is in contact with the hot air. Significant differences between half-piece and whole-pepper preparations may occur due to the same reason; differences on the availability of oxygen within the food tissues and loss of volatile constituents can either hinder or promote chemical and enzymatic reactions in the drying process (Lewicki, 2006).

A remarkable effect was also witnessed on chili peppers dried at 40 °C. Fantasia and Twilight showed substantial increase in capsaicinoids content when treated as whole fruits and by half-cut. Meanwhile, NA showed the same behaviour only for the first pre-treatment. Capsaicinoid increase in heat-treated chili peppers is not common, but has been previously reported (Ornelas-Paz et al., 2010; Yaldiz et al., 2010 ;Victoria-Campos et al., 2015). Victoria-Campos et al. (2015) analysed chilies treated by boiling and grilling, and suggested that capsaicinoids increase could be attributed to several factors such as dehydration of the food matrix, improved extractability of these compounds by cell disruption during thermal process, liberation of conjugated capsaicinoids, and inactivation of enzymes responsible for capsaicinoid breakdown such as peroxidases. Moreover, Toontom et al. (2012) tested a variety of *C. annuum L.* under hot air drying (60 °C), freeze-drying (-50 °C) and sun drying (37 °C), and reported statistically higher capsaicin contents for all the dried samples compared to raw chilies; though blanching pre-treatment of dried samples may have caused inactivation of peroxidase.

Trinidad Congo Yellow also responded positively to drying at 40 °C under the first two pre-treatments; although these variations were not significant in terms of total capsaicinoids, variations on capsaicin contents were statistically different. Dihydrocapsaicin did not have significant changes with regards to the drying processes in any of the chili peppers, differing from previous reports (Chinn et al., 2011; Topuz et al., 2011). Chinn et al. (2011) studied the influence of drying (oven and freeze-drying), on *C. chinense* pre-treated as whole peppers and separated by parts, results showed that capsaicin content in dry peppers was greater than in fresh peppers, while dihydrocapsaicin had an opposite behaviour, for specific pre-treatments. This indicates that variations on total capsaicinoids content may differ depending on the specific capsaicinoids type and the pre-treatment. Victoria-Campos et al., 2015 indicated that difference in thermostability among individual capsaicinoids may drive these changes.

7 CONCLUSIONS

The review of the nutritional value and the pungent principle of chili peppers allowed us to understand the importance of their use at household and industrial levels, and therefore the need to use the drying process for their preservation. The acknowledgement of possible changes on the flavour and nutritious qualities of chili peppers due to drying, encouraged us to assess the influence of this process on the final content of capsaicinoids in eleven varieties of chili peppers; furthermore, to determine the effects of different drying pre-treatments and drying temperatures on the final content of those capsaicinoids in the selected chili peppers.

Having achieved successful quantification of capsaicinoids on the different *Capsicum* varieties by means of GC-MS, tested three different pre-treatments and two drying temperatures, and quantified their influence on the content of the two major capsaicinoids through accurate statistical analysis, we can conclude that:

- The drying process had diverse effects on the final content of capsaicinoids depending on the variety of chili pepper. Capsaicinoid content remained stable in Hot Jalapeño, Black Plum and Timoito Pequeño. Meanwhile, contents in Habanero Bonda and Rubo Roso were importantly decreased ($p<0.05$) in general by the drying process.

- Drying chili peppers at 60 °C showed a general trend of decreasing capsaicinoids content, but only mashed chilies (Trinidad Congo Yellow, Rubo Roso, Habanero Bonda and NA) exhibited statistically significant differences.

- Drying at 40 °C displayed the outstanding effect of increasing the content of capsaicinoids in specific chili pepper varieties. Twilight and Fantasia in whole-fruit and half-cut preparations as well as NA in whole-fruit, exhibited significant improvements on final capsaicinoids content.

- Individual capsaicinoids behave differently regarding the drying process.

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APPENDIX A

a. One-way ANOVA statistical test results. Statistical values and probabilities per chili pepper variety and capsaicinoids.

CHILI PEPPER VARIETIES	Total Capsaicinoids		CAPSAICIN		DIHYDROCAPSAICIN	
	'F'	'Prob>F'	'F'	'Prob>F'	'F'	'Prob>F'
Hot Jalapeño	2.08E+00	1.22E-01	2.08E+00	**1.74E-02**	2.08E+00	2.57E-01
Cheyene Lutea	5.60E+00	**3.77E-03**	5.60E+00	2.50E-01	5.60E+00	**2.48E-02**
Black Plum	1.33E+00	3.09E-01	1.33E+00	**3.41E-02**	1.33E+00	3.19E-01
Fantasia	1.62E+01	**1.43E-05**	1.62E+01	**6.62E-11**	1.62E+01	5.17E-01
Timoito pequeño	3.25E+00	**3.23E-02**	3.25E+00	1.35E-01	3.25E+00	2.30E-01
Trinidad Congo Yellow	3.98E+01	**5.21E-08**	3.98E+01	**2.19E-08**	3.98E+01	**4.28E-02**
Rubo Roso	1.22E+01	**7.48E-05**	1.22E+01	**2.51E-06**	1.22E+01	1.40E-01
Habanero Bonda	1.44E+01	**2.83E-05**	1.44E+01	**9.51E-13**	1.44E+01	1.57E-01
Christmas Bells	3.59E+00	**2.28E-02**	3.59E+00	**2.35E-02**	3.59E+00	5.81E-02
Twilight	1.77E+01	**8.45E-06**	1.77E+01	**2.03E-04**	1.77E+01	5.36E-02
NA	1.32E+01	**4.68E-05**	1.32E+01	**2.34E-13**	1.32E+01	**4.50E-02**

b. Post hoc Tukey Honest Significant Difference ($p<0.05$) for total capsaicinoids in Cheyene Lutea.

CHEYENE LUTEA

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		2.84E-01	**3.78E-02**	1.98E-01	9.97E-01	9.98E-01	9.98E-01
Whole 40	2.84E-01		8.78E-01	1.00E+00	1.18E-01	5.57E-01	1.27E-01
Whole 60	**3.78E-02**	8.78E-01		9.52E-01	**1.36E-02**	9.73E-02	**1.48E-02**
Half 40	1.98E-01	1.00E+00	9.52E-01		7.84E-02	4.23E-01	8.48E-02
Half 60	9.97E-01	1.18E-01	**1.36E-02**	7.84E-02		9.18E-01	1.00E+00
Mash 40	9.98E-01	5.57E-01	9.73E-02	4.23E-01	9.18E-01		9.32E-01
Mash 60	9.98E-01	1.27E-01	**1.48E-02**	8.48E-02	1.00E+00	9.32E-01	

c. Post hoc Tukey Honest Significant Difference ($p<0.05$) for total capsaicinoids in Fantasia.

FANTASIA

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		**2.97E-02**	7.18E-01	**1.27E-03**	1.00E+00	4.43E-01	4.43E-01
Whole 40	**2.97E-02**		3.71E-01	5.96E-01	**1.50E-02**	**7.97E-04**	**7.97E-04**
Whole 60	7.18E-01	3.71E-01		**2.02E-02**	5.01E-01	**3.83E-02**	**3.83E-02**
Half 40	**1.27E-03**	5.96E-01	**2.02E-02**		**6.66E-04**	**4.80E-05**	**4.80E-05**
Half 60	1.00E+00	**1.50E-02**	5.01E-01	**6.66E-04**		6.58E-01	6.58E-01
Mash 40	4.43E-01	**7.97E-04**	**3.83E-02**	**4.80E-05**	6.58E-01		1.00E+00
Mash 60	4.43E-01	**7.97E-04**	**3.83E-02**	**4.80E-05**	6.58E-01	1.00E+00	

d. Post hoc Tukey Honest Significant Difference ($p<0.05$) for total capsaicinoids in Timoito Pequeño.

TIMOITO PEQUEÑO

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		1.93E-01	9.00E-01	9.38E-01	9.99E-01	7.68E-02	1.09E-01
Whole 40	1.93E-01		7.62E-01	6.93E-01	3.70E-01	9.97E-01	1.00E+00
Whole 60	9.00E-01	7.62E-01		1.00E+00	9.90E-01	4.47E-01	5.63E-01
Half 40	9.38E-01	6.93E-01	1.00E+00		9.96E-01	3.82E-01	4.91E-01
Half 60	9.99E-01	3.70E-01	9.90E-01	9.96E-01		1.63E-01	2.25E-01
Mash 40	7.68E-02	9.97E-01	4.47E-01	3.82E-01	1.63E-01		1.00E+00
Mash 60	1.09E-01	1.00E+00	5.63E-01	4.91E-01	2.25E-01	1.00E+00	

e. Post hoc Tukey Honest Significant Difference ($p<0.05$) for total capsaicinoids in Trinidad Congo Yellow.

TRINIDAD CONGO YELLOW

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		4.47E-01	**1.76E-05**	9.93E-01	6.08E-02	8.79E-01	**3.02E-06**
Whole 40	4.47E-01		**1.29E-06**	8.15E-01	**1.61E-03**	6.98E-02	**3.10E-07**
Whole 60	**1.76E-05**	**1.29E-06**		**7.10E-06**	**3.60E-03**	**1.06E-04**	8.33E-01
Half 40	9.93E-01	8.15E-01	**7.10E-06**		**1.86E-02**	5.29E-01	**1.35E-06**
Half 60	6.08E-02	**1.61E-03**	**3.60E-03**	**1.86E-02**		4.05E-01	**3.70E-04**
Mash 40	8.79E-01	6.98E-02	**1.06E-04**	5.29E-01	4.05E-01		**1.51E-05**
Mash 60	**3.02E-06**	**3.10E-07**	8.33E-01	**1.35E-06**	**3.70E-04**	**1.51E-05**	

f. Post hoc Tukey Honest Significant Difference ($p<0.05$) for total capsaicinoids in Rubo Roso.

RUBO ROSO

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		**1.89E-04**	**6.69E-04**	2.33E-01	**1.09E-04**	**1.14E-02**	**4.84E-03**
Whole 40	**1.89E-04**		9.85E-01	**1.44E-02**	1.00E+00	2.80E-01	5.14E-01
Whole 60	**6.69E-04**	9.85E-01		5.73E-02	9.14E-01	6.84E-01	9.09E-01
Half 40	2.33E-01	**1.44E-02**	5.73E-02		**7.64E-03**	6.06E-01	3.49E-01
Half 60	**1.09E-04**	1.00E+00	9.14E-01	**7.64E-03**		1.65E-01	3.35E-01
Mash 40	**1.14E-02**	2.80E-01	6.84E-01	6.06E-01	1.65E-01		9.99E-01
Mash 60	**4.84E-03**	5.14E-01	9.09E-01	3.49E-01	3.35E-01	9.99E-01	

g. Post hoc Tukey Honest Significant Difference ($p<0.05$) for total capsaicinoids in Habanero Bonda.

HABANERO BONDA

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		**2.94E-04**	**1.13E-04**	**7.23E-05**	**7.98E-05**	**6.51E-03**	**4.83E-05**
Whole 40	**2.94E-04**		9.96E-01	9.69E-01	9.78E-01	5.76E-01	9.00E-01
Whole 60	**1.13E-04**	9.96E-01		1.00E+00	1.00E+00	2.73E-01	9.97E-01
Half 40	**7.23E-05**	9.69E-01	1.00E+00		1.00E+00	1.76E-01	1.00E+00
Half 60	**7.98E-05**	9.78E-01	1.00E+00	1.00E+00		1.95E-01	1.00E+00
Mash 40	**6.51E-03**	5.76E-01	2.73E-01	1.76E-01	1.95E-01		1.14E-01
Mash 60	**4.83E-05**	9.00E-01	9.97E-01	1.00E+00	1.00E+00	1.14E-01	

h. Post hoc Tukey Honest Significant Difference ($p<0.05$) for total capsaicinoids in Christams Bells.

CHRISTMAS BELLS

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		6.46E-01	**4.26E-02**	1.37E-01	9.84E-01	9.99E-01	1.91E-01
Whole 40	6.46E-01		5.47E-01	8.97E-01	9.67E-01	8.64E-01	9.56E-01
Whole 60	**4.26E-02**	5.47E-01		9.92E-01	1.60E-01	8.93E-02	9.69E-01
Half 40	1.37E-01	8.97E-01	9.92E-01		4.23E-01	2.63E-01	1.00E+00
Half 60	9.84E-01	9.67E-01	1.60E-01	4.23E-01		1.00E+00	5.39E-01
Mash 40	9.99E-01	8.64E-01	8.93E-02	2.63E-01	1.00E+00		3.53E-01
Mash 60	1.91E-01	9.56E-01	9.69E-01	1.00E+00	5.39E-01	3.53E-01	

i. Post hoc Tukey Honest Significant Difference ($p<0.05$) for total capsaicinoids in NA.

NA

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		**2.02E-02**	6.06E-01	1.00E+00	8.16E-01	1.62E-01	**4.46E-02**
Whole 40	**2.02E-02**		3.62E-01	**2.44E-02**	2.09E-01	**1.76E-04**	**5.46E-05**
Whole 60	6.06E-01	3.62E-01		6.68E-01	1.00E+00	**7.50E-03**	**1.95E-03**
Half 40	1.00E+00	**2.44E-02**	6.68E-01		8.63E-01	1.37E-01	**3.69E-02**
Half 60	8.16E-01	2.09E-01	1.00E+00	8.63E-01		**1.50E-02**	**3.83E-03**
Mash 40	1.62E-01	**1.76E-04**	**7.50E-03**	1.37E-01	**1.50E-02**		9.86E-01
Mash 60	**4.46E-02**	**5.46E-05**	**1.95E-03**	**3.69E-02**	**3.83E-03**	9.86E-01	

j. Post hoc Tukey Honest Significant Difference ($p<0.05$) for Capsaicin in Hot Jalapeño.

HOT JALAPEÑO

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		9.99E-01	6.13E-02	9.99E-01	9.99E-01	1.00E+00	9.99E-01
Whole 40	9.99E-01		**2.78E-02**	1.00E+00	1.00E+00	1.00E+00	1.00E+00
Whole 60	6.13E-02	**2.78E-02**		**2.90E-02**	**2.78E-02**	**3.10E-02**	**2.75E-02**
Half 40	9.99E-01	1.00E+00	**2.90E-02**		1.00E+00	1.00E+00	1.00E+00
Half 60	9.99E-01	1.00E+00	**2.78E-02**	1.00E+00		1.00E+00	1.00E+00
Mash 40	1.00E+00	1.00E+00	**3.10E-02**	1.00E+00	1.00E+00		1.00E+00
Mash 60	9.99E-01	1.00E+00	**2.75E-02**	1.00E+00	1.00E+00	1.00E+00	

k. Post hoc Tukey Honest Significant Difference ($p<0.05$) for Capsaicin in Black Plum.

BLACK PLUM

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		7.27E-01	9.79E-01	4.51E-01	7.48E-01	1.77E-01	3.12E-01
Whole 40	7.27E-01		2.92E-01	9.99E-01	1.00E+00	9.03E-01	9.85E-01
Whole 60	9.79E-01	2.92E-01		1.39E-01	3.08E-01	**4.41E-02**	8.60E-02
Half 40	4.51E-01	9.99E-01	1.39E-01		9.98E-01	9.93E-01	1.00E+00
Half 60	7.48E-01	1.00E+00	3.08E-01	9.98E-01		8.89E-01	9.81E-01
Mash 40	1.77E-01	9.03E-01	**4.41E-02**	9.93E-01	8.89E-01		1.00E+00
Mash 60	3.12E-01	9.85E-01	8.60E-02	1.00E+00	9.81E-01	1.00E+00	

l. Post hoc Tukey Honest Significant Difference ($p<0.05$) for Capsaicin in Fantasia.

FANTASIA

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		**2.99E-05**	9.99E-01	**4.37E-08**	4.45E-01	**8.34E-05**	1.87E-01
Whole 40	**2.99E-05**		**6.10E-05**	**4.05E-05**	**7.26E-04**	**4.69E-08**	**9.64E-07**
Whole 60	9.99E-01	**6.10E-05**		**4.69E-08**	7.19E-01	**4.05E-05**	8.47E-02
Half 40	**4.37E-08**	**4.05E-05**	**4.69E-08**		**7.66E-08**	**3.77E-08**	**3.83E-08**
Half 60	4.45E-01	**7.26E-04**	7.19E-01	**7.66E-08**		**4.74E-06**	**5.29E-03**
Mash 40	**8.34E-05**	**4.69E-08**	**4.05E-05**	**3.77E-08**	**4.74E-06**		**7.21E-03**
Mash 60	1.87E-01	**9.64E-07**	8.47E-02	**3.83E-08**	**5.29E-03**	**7.21E-03**	

m. Post hoc Tukey Honest Significant Difference ($p<0.05$) for Capsaicin in Trinidad Congo Yellow.

TRINIDAD CONGO YELLOW

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		**2.25E-04**	**2.86E-04**	**4.78E-02**	9.99E-01	6.82E-01	4.43E-01
Whole 40	**2.25E-04**		1.00E+00	**1.75E-06**	**4.58E-04**	**1.97E-05**	2.10E-01
Whole 60	**2.86E-04**	1.00E+00		**2.10E-06**	**5.86E-04**	**2.43E-05**	2.04E-01
Half 40	**4.78E-02**	**1.75E-06**	**2.10E-06**		**2.19E-02**	5.47E-01	**4.81E-05**
Half 60	9.99E-01	**4.58E-04**	**5.86E-04**	**2.19E-02**		4.36E-01	6.58E-01
Mash 40	6.82E-01	**1.97E-05**	**2.43E-05**	5.47E-01	4.36E-01		1.00E+00
Mash 60	4.43E-01	2.10E-01	2.04E-01	**4.81E-05**	6.58E-01	1.00E+00	

n. Post hoc Tukey Honest Significant Difference ($p<0.05$) for Capsaicin in Rubo Roso.

RUBO ROSO

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		**1.55E-05**	**2.95E-05**	5.20E-02	**8.94E-06**	**1.01E-05**	**2.89E-04**
Whole 40	**1.55E-05**		9.99E-01	**3.59E-03**	1.00E+00	1.00E+00	4.95E-01
Whole 60	**2.95E-05**	9.99E-01		**8.08E-03**	9.75E-01	9.86E-01	7.53E-01
Half 40	5.20E-02	**3.59E-03**	**8.08E-03**		**1.77E-03**	**2.07E-03**	1.13E-01
Half 60	**8.94E-06**	1.00E+00	9.75E-01	**1.77E-03**		1.00E+00	2.97E-01
Mash 40	**1.01E-05**	1.00E+00	9.86E-01	**2.07E-03**	1.00E+00		3.37E-01
Mash 60	**2.89E-04**	4.95E-01	7.53E-01	1.13E-01	2.97E-01	3.37E-01	

o. Post hoc Tukey Honest Significant Difference (p<0.05) for Capsaicin in Habanero Bonda.

HABANERO BONDA

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		**3.83E-08**	**3.76E-08**	**3.76E-08**	**3.77E-08**	**3.95E-08**	**3.77E-08**
Whole 40	**3.83E-08**		**5.75E-07**	**2.72E-07**	5.99E-02	8.18E-01	**5.20E-03**
Whole 60	**3.76E-08**	**5.75E-07**		9.87E-01	**4.09E-05**	**1.59E-07**	**3.38E-04**
Half 40	**3.76E-08**	**2.72E-07**	9.87E-01		**1.41E-05**	**9.48E-08**	**1.03E-04**
Half 60	**3.77E-08**	5.99E-02	**4.09E-05**	**1.41E-05**		**5.20E-03**	8.18E-01
Mash 40	**3.95E-08**	8.18E-01	**1.59E-07**	**9.48E-08**	**5.20E-03**		**4.92E-04**
Mash 60	**3.77E-08**	**5.20E-03**	**3.38E-04**	**1.03E-04**	8.18E-01	**4.92E-04**	

p. Post hoc Tukey Honest Significant Difference (p<0.05) for Capsaicin in Christams Bells.

CHRISTMAS BELLS

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		1.00E+00	**3.41E-02**	1.00E+00	1.00E+00	1.00E+00	1.00E+00
Whole 40	1.00E+00		**4.42E-02**	1.00E+00	1.00E+00	1.00E+00	1.00E+00
Whole 60	**3.41E-02**	**4.42E-02**		6.03E-02	5.03E-02	**3.41E-02**	**3.41E-02**
Half 40	1.00E+00	1.00E+00	6.03E-02		1.00E+00	1.00E+00	1.00E+00
Half 60	1.00E+00	1.00E+00	5.03E-02	1.00E+00		1.00E+00	1.00E+00
Mash 40	1.00E+00	1.00E+00	**3.41E-02**	1.00E+00	1.00E+00		1.00E+00
Mash 60	1.00E+00	1.00E+00	**3.41E-02**	1.00E+00	1.00E+00	1.00E+00	

q. Post hoc Tukey Honest Significant Difference (p<0.05) for Capsaicin in Twilight.

TWILIGHT

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		1.00E+00	1.00E+00	**4.68E-04**	1.00E+00	9.97E-01	1.00E+00
Whole 40	1.00E+00		9.99E-01	**9.01E-04**	1.00E+00	1.00E+00	9.89E-01
Whole 60	1.00E+00	9.99E-01		**4.01E-04**	9.99E-01	9.94E-01	1.00E+00
Half 40	**4.68E-04**	**9.01E-04**	**4.01E-04**		**8.55E-04**	**1.19E-03**	**2.73E-04**
Half 60	1.00E+00	1.00E+00	9.99E-01	**8.55E-04**		1.00E+00	9.92E-01
Mash 40	9.97E-01	1.00E+00	9.94E-01	**1.19E-03**	1.00E+00		9.71E-01
Mash 60	1.00E+00	9.89E-01	1.00E+00	**2.73E-04**	9.92E-01	9.71E-01	

r. Post hoc Tukey Honest Significant Difference (p<0.05) for Capsaicin in NA.

NA

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		**3.80E-08**	**4.78E-07**	**8.90E-03**	**2.22E-04**	**4.28E-07**	**5.81E-06**
Whole 40	**3.80E-08**		**3.76E-08**	**3.77E-08**	**1.32E-07**	**3.76E-08**	**3.76E-08**
Whole 60	**4.78E-07**	**3.76E-08**		**1.58E-04**	**3.86E-08**	1.00E+00	4.05E-01
Half 40	**8.90E-03**	**3.77E-08**	**1.58E-04**		**5.99E-07**	**1.33E-04**	**5.67E-03**
Half 60	**2.22E-04**	**1.32E-07**	**3.86E-08**	**5.99E-07**		**3.85E-08**	**4.33E-08**
Mash 40	**4.28E-07**	**3.76E-08**	1.00E+00	**1.33E-04**	**3.85E-08**		3.53E-01
Mash 60	**5.81E-06**	**3.76E-08**	4.05E-01	**5.67E-03**	**4.33E-08**	3.53E-01	

s. Post hoc Tukey Honest Significant Difference ($p<0.05$) for Dihydroapsaicin in Cheyene Lutea.

CHEYENE LUTEA

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		2.11E-01	1.21E-01	1.37E-01	1.00E+00	5.22E-01	9.99E-01
Whole 40	2.11E-01		1.00E+00	1.00E+00	2.19E-01	9.92E-01	3.99E-01
Whole 60	1.21E-01	1.00E+00		1.00E+00	1.25E-01	9.39E-01	2.46E-01
Half 40	1.37E-01	1.00E+00	1.00E+00		1.43E-01	9.58E-01	2.77E-01
Half 60	1.00E+00	2.19E-01	1.25E-01	1.43E-01		5.34E-01	9.99E-01
Mash 40	5.22E-01	9.92E-01	9.39E-01	9.58E-01	5.34E-01		7.78E-01
Mash 60	9.99E-01	3.99E-01	2.46E-01	2.77E-01	9.99E-01	7.78E-01	

t. Post hoc Tukey Honest Significant Difference ($p<0.05$) for Dihydroapsaicin in Trinidad Congo Yellow.

TRINIDAD CONGO YELLOW

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		9.93E-01	2.67E-01	1.68E-01	8.98E-02	1.28E-01	9.12E-01
Whole 40	9.93E-01		6.01E-01	4.30E-01	2.59E-01	3.47E-01	9.99E-01
Whole 60	2.67E-01	6.01E-01		1.00E+00	9.92E-01	9.99E-01	8.48E-01
Half 40	1.68E-01	4.30E-01	1.00E+00		1.00E+00	1.00E+00	6.92E-01
Half 60	8.98E-02	2.59E-01	9.92E-01	1.00E+00		1.00E+00	4.75E-01
Mash 40	1.28E-01	3.47E-01	9.99E-01	1.00E+00	1.00E+00		5.95E-01
Mash 60	9.12E-01	9.99E-01	8.48E-01	6.92E-01	4.75E-01	5.95E-01	

u. Post hoc Tukey Honest Significant Difference ($p<0.05$) for Dihydroapsaicin in NA.

NA

	Fresh	Whole 40	Whole 60	Half 40	Half 60	Mash 40	Mash 60
Fresh		8.71E-01	4.31E-01	8.27E-01	1.00E+00	1.00E+00	9.14E-01
Whole 40	8.71E-01		6.39E-02	2.10E-01	9.78E-01	7.31E-01	1.00E+00
Whole 60	4.31E-01	6.39E-02		9.89E-01	2.48E-01	5.93E-01	7.87E-02
Half 40	8.27E-01	2.10E-01	9.89E-01		6.05E-01	9.35E-01	2.51E-01
Half 60	1.00E+00	9.78E-01	2.48E-01	6.05E-01		9.91E-01	9.90E-01
Mash 40	1.00E+00	7.31E-01	5.93E-01	9.35E-01	9.91E-01		7.94E-01
Mash 60	9.14E-01	1.00E+00	7.87E-02	2.51E-01	9.90E-01	7.94E-01	