Food Chemistry 238 (2018) 51–57

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Capsaicinoids, amino acid and fatty acid profiles in different fruit components of the world hottest Naga king chilli (*Capsicum chinense* Jacq)

R. Ananthan *, K. Subhash, T. Longvah

National Institute of Nutrition, Jamai Osmania (PO), Hyderabad 500 007, Telangana State, India

ARTICLE INFO

Article history:
Received 31 July 2016
Received in revised form 21 November 2016
Accepted 20 December 2016
Available online 23 December 2016

Keywords:
Naga king chilli
Capsicum chinense
Fruit components
Fatty acids
Capsaicinoids

ABSTRACT

The world hottest Naga king chilli is cultivated and consumed in Northeast India. Capsaicinoids, amino acids and fatty acids were studied in fruit and fruit components of Naga king chilli. Capsaicinoid content was increased in each ripening stage and maximum level was observed at red color fruits. Total protein and fat content of placenta was 19.41 and 20.36% respectively. Capsaicinoids of placenta (7.35 ± 2.241%) was higher followed by seed (3.83 ± 1.358%) and pericarp (2.91 ± 0.667%). Similarly, essential amino acid content was also higher in placenta compared to other components. Amino acid score ranged from 37 to 38 with cystine and methionine as limiting amino acid. Low level of palmitic, stearic and α-linolenic acid and very high level of linoleic acid were found in seeds. Total polyunsaturates of seeds were higher followed by whole fruit. Naga king chilli is unique due to its high capsaicinoid content and it offers potential crop for the future exploitation.

1. Introduction

Red chillies (*Capsicum* sp.) belong to the Solanaceae plant family and the genus *Capsicum* comprising many species, of which only three species such as *C. annuum, C. frutescens,* and *C. chinense* are widely cultivated. Red chillies are the most cultivated and consumed spice worldwide, particularly in temperate and semi-temperate countries. Red chillies also contain many nutrients including minerals, vitamins, phenolic acids and flavonoids which are bioactive health promoting components of human diet (Ananthan, Subash, & Longvah, 2014; Marín, Ferreres, Tomás-Barberán, & Gil, 2004; Ogunlade, Alebiosu, & Osasona, 2012). Depending on the flavoring intensity, shape, size, color and texture, chilli fruits are used in various culinary preparations during its different developmental stages. Red chillies are not only valued for their sensory attributes of color, aroma and pungency, but also have significant role in pharmaceuticals applications. The pungent flavor of red chilli is due to presence of group of acid amides collectively called as capsaicinoids produced by a condensation reaction between an aromatic vanillylamine moiety and a C9-C11 branched-chain fatty acid (Díaz, Pomar, Bernal, & Merino, 2004; Sukrasno & Yeoman, 1993). Synthesis of capsaicinoids is initiated by amino acids, phenylalanine and valine, which form the fatty acid and vanillylamine moieties respectively by phenylpropanoid pathway (Baas-Espinola, Castro-Concha, Vázquez-Flota, & Miranda-Ham, 2016; Bennett & Kirby, 1968). Capsaicin (rans-8-methyl-*N*-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-Nvanillylnonanamide) are the major capsaicinoids in chillies, and contribute almost 90% of total pungency of red chillies (Gnayfeed, Daood, Biacs, & Alcaraz, 2001; Govindarajan, Rajalakshmi, Chand, & Salzer, 1987).

Capsaicinoids are synthesized and accumulated in the epidermal tissue of placenta during fruit development (Fujiwake, Suzuki, & Iwai, 1980). Capsaicinoids are also found in other parts of the fruit such as pericarp and seed (Conforti, Statti, & Menichini, 2007; Sukrasno & Yeoman, 1993). Accumulation of capsaicinoids increases during the fruit ripening from green to red color stage. It has been reported that the capsaicinoids accumulation and pungency of chillies are influenced both by genetic and environmental factors such as the ripening stage of the fruit, species, cultivar and agro-climatic conditions (Barbero et al., 2014; Contreras-Padilla & Yahia, 1998; Zewdie & Bosland, 2000).

India is the largest chilli cultivating country in the world and produced 1,376,000 tonnes during 2013 (FAOSTAT, 2016). In Northeast India, chillies represent a culinary tradition and cultural identity, where many land races of chilli varieties are grown and consumed by the local population. Among them, Naga king chilli (*Capsicum chinense* Jacq.), conventionally used by the Tangkhul

* Corresponding author at: Food Chemistry Division, National Institute of Nutrition, Jamai Osmania (PO), Hyderabad 500 007, Telangana State, India.
E-mail address: ananthan.nin@gmail.com (R. Ananthan).

http://dx.doi.org/10.1016/j.foodchem.2016.12.073
0308-8146/

tribe in Manipur is now used and cultivated by the various ethnic groups all over the Northeast India. Naga king chilli has recently received world attention, due to its extremely high pungency and unique aroma. It has also been acknowledged as the world hottest chilli, measuring 1001304 Scoville Heat Unit (SHU). Apart from being a vegetable and spice crop, Naga king chilli is unique due to its high capsaicinoid content thereby presenting itself as a potential crop for the future exploitation in food and pharmaceutical industries. However, wide genetic variability has been reported among the Naga king chilli landraces (Bhagowati & Changkija, 2009; Islam et al., 2015) and there is no comprehensive data on fatty acid and amino acid profiles and capsaicinoid content of the fruit and its components. In the study we investigated the capsaicinoid content at different ripening stages of the fruit maturity, as well as amino acid and fatty profile in the different fruit components of Naga king chilli.

2. Materials & methods

2.1. Sample collection

Fresh Naga king chillies were collected at three different stages of the fruit maturity *viz.* green, yellow and red fruits from Chatrick village of Ukhrul district in Manipur. Dried Naga king chillies weighing around 2.0 kg were collected from 15 different locations from the states of Nagaland, Manipur and one sample from Arunachal Pradesh, and transported to the laboratory at Hyderabad.

2.2. Standards and chemicals

Amino acid standard mixtures were purchased from Agilent (Agilent Technologies, Switzerland) and fatty acid standards were procured from Nu-Chek, USA. Capsaicin (>97%) and dihydrocapsaicin (>90%) were purchased from Sigma (St. Louis, MO). HPLC grade acetonitrile, methanol, and analytical grade formic acid were procured from Merck (Merck, India). All other chemicals were of analytical grade purity. HPLC solvents were filtered through 0.22 μm membrane filter (Millipore, HVHP04700) and degassed in an ultrasonic bath before use.

2.3. Sample preparation

Whole fruits of the Naga king chilli samples were cut into two halves vertically and the fruit components such as seed, placenta and pericarp were separated and minced individually into fine paste (fresh samples) using mortar and pestle or powder (dried samples) using a domestic mixie. Whole fruit was also minced separately. Aliquots were taken for analysis of capsaicinoids, amino acids and fatty acids content of whole fruit and its components.

2.4. Sample extraction for capsaicinoids determination

Several methods using different solvents, temperature and extraction time have been reported for the extraction and analysis of capsaicinoids (Choi et al., 2006; Contreras-Padilla & Yahia, 1998; Garcés-Claver, Arnedo-Andrés, Abadía, Gil-Ortega, & Álvarez-Fernández, 2006; Kozukue et al., 2005). Therefore, to select the most suitable method for maximum recovery, extraction was carried out with three individual organic solvents namely methanol, ethanol and acetonitrile at different temperature and extraction was also carried out by ultrasonic bath at different length of time. 500 mg of sample was transferred into 30 mL screw cap tubes, mixed with individual organic solvents and the volume was made up to approximately 10 mL with respective solvent. The sample tube was incubated at two different temperatures (70 °C or 50 °C) for 5 and 3 h. Separate extraction was also carried out in individual solvents by ultrasound sonication for 1 or 2 h. After the specified extraction procedure, the tubes were brought to room temperature. Final volume was adjusted to 25 mL with respective solvent and centrifuged (Sigma, Germany) at 4000 rpm for 10 min at 4 °C. The supernatant was collected, filtered through a 0.45 μm syringe nylon filter (Millipore, Bedford, MA) and used for analysis.

2.5. HPLC analysis of capsaicinoids and calculation of Scoville Heat Unit

Capsaicinoid analogues were analysed using HPLC (Agilent-1100; Agilent technologist, USA) equipped with auto-sampler and UV–VIS detector. A Symmetry C_{18} column (250 × 4.6 mm) packed with 5 μm particle size (Waters; WAT 054275) was used at ambient temperature and the injection volume of 20 μL was delivered through an auto-sampler. Isocratic flow (1 mL/min) of 60% (v/v) acetonitrile prepared in 0.5% (v/v) of formic acid was maintained at ambient temperature and the compounds were detected by UV detector at 280 nm. The concentration of the individual capsaicinoid was quantified using external standard calibration plotted with five different concentrations [0–10 μg/mL]. Confirmation of the capsaicinoid analogues was carried out by Thermo HPLC (Spectra system 2000) attached to Thermo finnigan (LCQ Advantage MAX) Ion-trap Mass Spectrometer (Thermo finnigan, San Jose, CA). HPLC separation conditions were maintained as described above. For optimum Mass Spectrometer (MS) results, ionization was performed in positive ESI mode. Temperature of nebulizer, spray voltage and flow of the MS were set to 300 °C, 5 kV and 40 psi (nitrogen) respectively; mass scan range was set in the range of *m*/*z* 200–400. Scoville Heat Unit (SHU) was calculated based on the standard values of pure capsaicin (16,000,000/100 g), dihydrocapsaicin (15,000,000/100 g) and nordihydrocapsaicin (9,100,000/100 g).

2.6. Protein and amino acid determination

Crude protein (AOAC, 2006; 984.13) was estimated (N x 6.25) from total nitrogen, which was determined by automated nitrogen analyzer (FOSS Kjeltec, Sweden). Amino acid analysis was carried out by hydrolyzing 10 mg protein equivalent of the sample with 6 N HCl in sealed ampoules in an oven (Biotechnics, India) at 110 °C for 22 h (Darragh, 2005). Excess acid was removed by continuous flash evaporation under reduced pressure (Buchi, Switzerland) and the sample was then dissolved in citrate buffer (pH 2.2). An aliquot (20 μL) of the sample was loaded into the automated amino acid analyzer (Biochrom-30, Cambridge, UK). Methionine and cysteine were determined separately, after converting them into methionine sulfone and cysteic acid respectively, using performic acid oxidation (Moore, 1963). Tryptophan was quantified after barytic hydrolysis according to method described by Landry and Delhaye (1992). Each amino acid was identified and quantified using authentic standard (National Institute of Standards and Technology, SRM 2389) and expressed in milligram (mg) per 100 g of protein. Amino acid score was calculated with reference to the pattern of amino acids requirement for preschool children (2–5 years) suggested by FAO/WHO/UNU (1985).

2.7. Fat and fatty acid determination

Total fat content was quantified using Association of Official Analytical Chemists methods (2003.05) by solvent extraction using chloroform:methanol (2:1) as extractant (AOAC, 2006) The fatty acid analysis was carried out by preparing fatty acid methyl esters (FAME) of the samples according to the method of O'Fallon,