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The influence of fruit ripening on the phytochemical content and biological activity of *Capsicum chinense* Jacq. cv Habanero

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ABSTRACT

During the past decade, it has been reported that the consumption of certain foods and spices such as pepper may have a positive effect on health. The present study evaluates the influence of fruit ripening on total phenols, flavonoids, carotenoids and capsaicinoids content and antioxidant, hypoglycaemic and anticholinesterase activities of *Capsicum chinense* Jacq. cv Habanero. The chemical investigation showed a different composition between the two stages of ripening (immature and mature). Generally, the concentration of carotenoids and capsaicinoids increased as the peppers reached maturity, whereas the concentration of phenols declined. The immature fruits showed the highest radical scavenging activity (IC_{50} of 97.14 μg/ml). On the contrary, the antioxidant activity evaluated by the β-carotene bleaching test showed a significant activity for mature peppers (IC_{50} value of 4.57 μg/ml after 30 min of incubation). Mature peppers inhibited α-amylase with an IC_{50} of 130.67 μg/ml. The lipophilic fractions of both mature and immature peppers exhibited an interesting and selective inhibitory activity against α-amylase with IC_{50} values of 29.58 and 9.88 μg/ml, respectively. Both total extracts of mature and immature peppers inhibited butyrylcholinesterase selectively. The obtained results underline the potential health benefits as a result of consuming *C. chinense* Habanero and suggest that it could be used as new valuable flavour with functional properties for food or nutriceutical products on the basis of the high content of phytochemicals and found biological properties.

1. Introduction

A wealth of information and scientific evidence is rapidly accumulating that shows the beneficial effects of a wide variety of food components on human health. In the past few years, there has been a renewed interest in studying and quantifying the antioxidant constituents of fruits and vegetables for their potential health functionality against various diseases such as diabetes, cancer, cardiovascular and neurodegenerative diseases such as Alzheimer's disease (Kaur & Kapoor, 2001). Diabetes is a major risk factor for premature atherosclerosis and oxidative stress plays an important role in its pathogenesis (Venugopal, Devaraj, Yang, & Jialal, 2002). One therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise. Oxidative stress has been described in the pathological changes that occur in Alzheimer's disease (AD), the most common form of neurodegenerative disorders (Pratico & Delanty, 2000). AD is currently treated clinically by the use of agents which restore the level of acetylcholine through inhibition of both two major forms of cholinesterase: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) (Loizzo, Tundis, Menichini, & Menichini, 2008).

The genus *Capsicum*, which originates from tropical and humid zones of Central and Southern America, belongs to the Solanaceae family and includes peppers of important economic value. Several *Capsicum* species exist, three of which are widely spread and have a hot or pungent berry: *Capsicum annuum*, *Capsicum frutescens* and *Capsicum chinense*. The Habanero chili pepper is the fruit of *C. chinense* Jacq., a very aromatic variety and is claimed to be the hottest chili pepper in the world. It is of great interest to know the contribution of an individual food product in the daily nutritional needs and how ripening affect dietary, nutrition and therefore biological properties. The content of phytochemicals in plant material is influenced by numerous factors such as climatic

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conditions, ripening time, genotype and cultivation techniques. The maturity stage is another important factor that influences the compositional quality of fruit and vegetables, since, during fruit ripening, several biochemical, physiological and structural modifications happen and these changes determine the attributes of fruit quality.

In relation to the importance of phytochemicals and antioxidant power for functional aspects of pepper, the aim of this work was to evaluate the influence of ripening stage on several quality attributes, such as total carotenoids, phenols and capsaicinoids. To this purpose, the antioxidant activity, the inhibition of the two major carbohydrate digestive enzymes, α-amylase and α-glucosidase, and the inhibition of two enzymes frequently targeted for the treatment of Alzheimer's disease were investigated and were related to the different chemical composition of *C. chinense* Habanero, widely consumed in diets, in the two stages of ripening.

2. Materials and methods

2.1. Chemicals

Methanol, ethanol, ethyl acetate, *n*-hexane, sodium sulfate, DMSO, H_2SO_4, chloroform, sodium carbonate, perchloric acid, HCl, KOH, NaOH, NaCl, butanol, thin-layer chromatography plates (TLC) were obtained from VWR International s.r.l. (Milano, Italy). Capsaicin, dihydrocapsaicin, β-carotene, quercetin, chlorogenic acid, thiobarbituric acid (TBA), phosphate buffered saline (PBS), bovine brain extract, $FeCl_3$, $AlCl_3$, NaCl, ascorbic acid, *o*-dianisine (DIAN), peroxidase/glucose oxidase (PGO) system, butylated hydroxytoluene (BHT), propyl gallate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), linoleic acid, Tween 20, Folin–Ciocalteu reagent, potato starch, sodium phosphate buffer, sodium chloride, sodium potassium tartrate tetrahydrate, 3,5-dinitrosalicylic acid, maltose, α-amylase (EC 3.2.1.1), α-glucosidase (EC 3.2.1.20), acetylthiocholine iodide (ATCI), 5,5′-dithiobis(2-nitrobenzoic-acid) (DTNB), butyrylthiocholine iodide (BTCI), physostigmine, acetylcholinesterase from *Electrophorus electricus* (EC 3.1.1.7, Type VI-S) and butyrylcholinesterase from equine serum (EC 3.1.1.8) were purchased from Sigma–Aldrich S.p.a. (Milano, Italy). Acarbose from *Actinoplanes* sp. was obtained from Serva (Heidelberg, Germany).

2.2. Plant materials

The fruits of *C. chinense* Jacq. cv Habanero used in this study were purchased in September 2006 from farm Miceli s.r.l. (Calabria, Italy). The peppers were harvested at the same time but at two successive maturity stage on the basis of their colour as immature or green (I) and mature or red (M). The authentication of the pepper was carried out at Natural History Museum of Calabria and Botanic Garden, University of Calabria, Italy. All peppers received similar water and fertilizer treatments. Pepper fruits were examined for integrity and absence of dust and insect contamination, were devoid of peduncles and seeds and they were cut into small pieces. Samples were freeze-dried and stored at −20 °C until they were analysed.

2.3. Extraction procedure

Components in peppers were extracted from 200 g of fruits by ethanol. Extraction was repeated until the complete exhaustion of colour. The ethanol solutions were combined and dried over anhydrous Na_2SO_4 (total extract), obtaining a yield of 3.7% and 6.5% for immature and mature fruits, respectively. In order to operate a separation of lipophilic compounds, the total extract was solubilised with MeOH/H_2O (8:2) and extracted with *n*-hexane. The *n*-hexane solutions were combined and dried over anhydrous Na_2SO_4 to obtain the lipophilic fraction (yield 0.81% and 0.88% for immature and mature fruits, respectively).

2.4. Determination of total phenol and flavonoid content

The amount of total phenolics of pepper samples was determined by the Folin–Ciocalteu method (Gao, Ohlander, Jeppsson, Björk, & Trajkovski, 2000). Briefly, the extract was mixed with 0.2 ml Folin–Ciocalteu reagent (Sigma–Aldrich), 2 ml of distilled water and 1 ml of 15% Na_2CO_3. The absorbance was measured at 765 nm using a Jenway 6003 UV–Vis spectrophotometer after 2 h incubation at room temperature. The levels of total phenolics content were determined in triplicate. Chlorogenic acid was used as a standard and the total phenolics content was expressed as chlorogenic acid equivalents in mg *per* 100 g of fresh materials.

The flavonoids content was determined spectrophotometrically using a method based on the formation of a flavonoid–aluminium complex (Yoo, Lee, Lee, Moon, & Lee, 2008). One millilitre of the extracts was added to a 10 ml volumetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.6 ml of 10% (w/v) $AlCl_3$ was added and then at 6 min 2 ml of 1 M NaOH were also added to the mixture, followed by the addition of 2.1 ml distilled water. Absorbance at 510 nm was read immediately. Quercetin was chosen as a standard and the levels of total flavonoid content were determined in triplicate and expressed as quercetin equivalents in mg *per* 100 g fresh materials.

2.5. Determination of total carotenoid content

The total carotenoid content was determined by measuring the absorption of lipophilic fractions at 450 nm. Triplicate aliquots of fractions were analysed in a Jenway 6003 UV–Vis spectrophotometer. A 1 ml aliquot from the lipophilic layer (0.1 g/ml) was added to 0.5 ml of 5% NaCl, vortexed for 30 s and centrifuged for 10 min at 4500 rpm. The supernatant (100 μl) was diluted with 0.9 ml of *n*-hexane and measured at 460 nm. β-Carotene was used as a standard. The total carotenoids contents were determined in triplicate and expressed as β-carotene equivalents in mg *per* 100 g of fresh materials (Gao et al., 2000).

2.6. Determination of capsaicin and dihydrocapsaicin contents

Capsaicinoid content was evaluated by gas chromatography (GC). GC analyses were performed on a Shimadzu GC17A gas chromatograph equipped with a flame ionisation detector (FID) and controlled by Borwin Software. The samples were analysed on a fused silica 30 m SE-30 capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25 μm. Nitrogen was used as the gas vector at a constant flow of 1.0 ml/min. The injector and detector temperatures were 250 °C and 280 °C, respectively. Analyses were performed in isothermal conditions at 210 °C.

Quantitative data were obtained from the electronic integration of the GC peak areas for three injections of each sample with the use of commercial capsaicin and dihydrocapsaicin as external standards injected into the GC equipment under identical conditions as above. A stock solution of capsaicin and dihydrocapsaicin was prepared with acetone at a concentration of 0.05 g/5 ml. From this stock, six 1-ml solutions were prepared to be used to obtain a standard curve of the two capsaicinoids. The results were expressed as μg *per* g fresh weight.

2.7. GC–MS analysis

The *C. chinense* Habanero *n*-hexane fractions were analysed by gas chromatography–mass spectrometry (GC–MS). GC–MS