(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Food Research International 45 (2012) 170–176

Contents lists available at SciVerse ScienceDirect

Food Research International

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

Air-dried *capsicum annuum* var. *acuminatum* medium and big: Determination of bioactive constituents, antioxidant activity and carbohydrate-hydrolyzing enzymes inhibition

Rosa Tundis [a], Monica R. Loizzo [a,*], Federica Menichini [b], Marco Bonesi [a], Filomena Conforti [a], Damiano De Luca [c], Francesco Menichini [a]

[a] Department of Pharmaceutical Sciences, Faculty of Pharmacy and Nutrition and Health Sciences, University of Calabria, I-87030 Rende (CS) Italy
[b] AIFA, Italian Medicines Agency, I-00187 Roma, Italy
[c] CALAB, Laboratorio Chimico Merceologico della Calabria, I-87040 Montalto Uffugo (CS), Italy

ARTICLE INFO

Article history:
Received 27 July 2011
Accepted 19 October 2011
Available online 28 October 2011

Keywords:
Air-dried pepper
Flavonoids
HPLC
Carotenoids
Capsaicinoids
Antioxidant and hypoglycemic activities

ABSTRACT

In the present study the total phenols, flavonoids, carotenoids and capsaicinoids content, the *in vitro* antioxidant and hypoglycemic activities of extracts of air-dried fruits from two *Capsicum annuum* cultivars were investigated. A different composition between the two cultivars was evidenced. *C. annuum* var. *acuminatum* medium extract presented a major content of phenols, carotenoids, capsaicin and dihydrocapsaicin while *C. annuum* var. *acuminatum* big extract is characterized by the highest quercetin, luteolin and kaempferol content with 68.0, 87.6 and 29.7 μg/g dried weight, respectively, analyzed by HPLC. Medium pepper showed the highest radical scavenging activity in DPPH assay (IC_{50} of 85.3 μg/ml) while big pepper have an interesting activity in ABTS assay (IC_{50} of 16.4 μg/ml) and the highest inhibition of linoleic acid oxidation with an IC_{50} value of 1.2 μg/ml after 30 min of incubation. A selective inhibitory activity against α-amylase was demonstrated for *C. annuum* var. *acuminatum* big lipophilic fraction (IC_{50} values of 8.7 μg/ml). The obtained results suggest that *C. annuum* cultivars could be used as valuable flavor with functional properties for foods.

1. Introduction

The Mediterranean diet was found to be characteristically plant based, including fruit, vegetables, cereals and spice. Population-wide average dietary intake of common spices including hot red peppers (*Capsicum annuum* L.) has been estimated at 0.5 g/person per day in Europe (Fowles, Mitchell, & McGrath, 2001). The mature green fruits are roasted and consumed whole or added to enchiladas and salsas. The dried red fruits are used as seasonings or natural red colorant. Peppers are a remarkable source of antioxidants including flavonoids, phenolic acids and carotenoids (Howard, Talcott, Brenes, & Villalon, 2000; Lee, Howard, & Villalon, 1995; Matsufuji, Nakamura, Chino, & Takeda, 1998; Osuna-Garcia, Wall, & Waddell, 1998).

Reactive oxygen species (ROS) are involved in the pathogenesis of several human diseases and there is growing evidence that consumption of certain foods, dietary supplements and traditional beverages leads to a reduction in some parameters of oxidative damage in biological systems. The burst of ROS is considered one of the key factors also in the development of diabetes.

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia and alterations in carbohydrate, lipid and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and/or insulin action. The World Health Organization (WHO) estimates that more than 220 million people worldwide have diabetes and this number is likely to more than double by 2030 (WHO, 2009). The high prevalence of diabetes as well as its long-term complications has led to an ongoing search for hypoglycaemic agents from natural sources (Nicasio-Torres, Erazo-Gómez, & Cruz-Sosa, 2009). One therapeutic approach to treat the early stage of diabetes is to decrease 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. Consequently, inhibitors of these enzymes determine a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise (Tundis, Loizzo, Statti, & Menichini, 2007).

Several works demonstrated the ability of pepper and their constituents to influence the blood glucose level (Ahuja, Robertson, Geraghty, & Ball, 2006; Chaiyasit, Khovidhunkit, & Wittayalertpanya, 2009; Loizzo, Tundis, Menichini, Statti, & Menichini, 2008; Menichini et al., 2009; Oboh, Ademiluyi, & Faloye, 2011; Ranilla, Kwon, Apostolidis, & Shetty, 2010; Tolan, Ragoobirsingh, & Morrison, 2004; Tundis et al., 2011).

In our continuous search on antioxidant and hypoglycaemic plants food and spice, the aim of this study is to investigate the total phenols,

* Corresponding author. Fax: +39 984 493298.
E-mail address: mr.loizzo@unical.it (M.R. Loizzo).

0963-9969/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2011.10.028

carotenoids and capsaicinoids (capsaicin and dihydrocapsaicin) content and the HPLC flavonoids (quercetin, kaempferol, luteolin) quantification of two *C. annuum* cultivars (*acuminatum* medium and *acuminatum* big) fruits.

These cultivars were largely used after air-drying process in order to prepare a spicy powder used in traditional gastronomy to flavour typical dishes. Moreover, the antioxidant activities through different *in vitro* tests and the hypoglycaemic properties through α-amylase and α-glucosidase enzymes inhibition were tested and correlated to the chemical composition.

2. Methods and materials

2.1. Chemicals and reagents

The following chemicals were obtained from Sigma-Aldrich S.p.a. (Milano, Italy): quercetin, luteolin, kaempferol, linoleic acid, chlorogenic acid, capsaicin, dihydrocapsaicin, potato starch, sodium phosphate, sodium chloride, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulphate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), α-amylase from porcine pancreas (EC 3.2.1.1), α-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), maltose, sodium acetate, sodium potassium tartrate, 3,5-dinitrosalicylic acid, *o*-dianisidine color reagent (DIAN), glucose oxidase peroxidase enzyme solution (PGO), Folin-Ciocalteau reagent, β-carotene, anhydrous sodium sulphate, ascorbic acid, propyl gallate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Tween 20. Ethanol, methanol, chloroform, *n*-hexane, acetic acid, perchloric acid, sulphuric acid, DMSO, basic bismuth nitrate, potassium iodide, phosphoric acid, NaH_2PO_4 buffer, acetonitrile were purchased from VWR International s.r.l. (Milan, Italy). Acarbose was obtained from Serva (Heidelberg, Germany).

2.2. Plant material and extraction

Commercial pepper cultivars (*C. annuum* var. *acuminatum* medium and big) used in this study were purchased in September 2006 from farm Miceli s.r.l. (Italy). Fruits were harvested from a random sample of 20 plants in order to obtain a set of peppers that are representative for both cultivars. The authentication of samples was carried out by Dr. N. Passalacqua, Head of Herbarium Department at Natural History Museum of Calabria, University of Calabria, Italy. All varieties received similar water and fertilizer treatments. Red peppers were harvested at full fruit size at maturity. The *C. annuum* var. *acuminatum* cultivars are characterized by a length of 9 cm for medium pepper and 13 cm for big pepper both at the maturity stage. Peppers were examined for integrity and absence of dust and insect contamination, were devoid of peduncles and seeds, were cut into small pieces (about 1 cm), and were air-dried until analysis (28–30 °C for 2 weeks).

Soluble components in peppers were extracted from 200 g of fruits by ethanol (350 ml). Extraction was repeated until the complete exhaustion of color. The ethanol solutions were combined and dried to obtain the total extract.

Air-dried peppers (200 g) were extracted by ethanol to obtain the total extract (yield 5.4% and 6.2% for *C. annuum* var. *acuminatum* medium and *C. annuum* var. *acuminatum* big, respectively). In order to operate a separation of non polar compounds, ethanol extract was solubilized with MeOH/H_2O (8:2) and extracted with *n*-hexane to obtain a lipophilic fraction (yield 0.6% and 0.3% for *C. annuum* var. *acuminatum* medium and *C. annuum* var. *acuminatum* big, respectively).

2.3. Capsaicin and dihydrocapsaicin content

The GC analyses were performed on a Shimadzu GC17A gas chromatograph equipped with a flame ionization detector (FID) and controlled by Borwin Software. Samples were analysed on a fused silica capillary column SE-30 (30 m length; 0.25 mm i.d.; 0.25 μm film thickness). Nitrogen was used as the gas vector at a constant flow of 1.0 ml/min. Analyses were performed in isothermal conditions at 210 °C. The injector and detector temperatures were 250 °C and 280 °C, respectively. Diluted samples (1/100, v/v in *n*-hexane) of 1 μl was injected manually and in the splitless mode.

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 dried weight (dw).

2.4. Total carotenoid content

The total carotenoid content was determined by measuring the absorption of lipophilic fractions. Triplicate aliquots of fractions were analyzed spectrophotometrically in a UV–vis Jenway 6003 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 $\lambda = 460$ nm. β-Carotene was used as a standard. The total carotenoids content was determined in triplicate and expressed as β-carotene equivalents in mg *per* 100 g dw (Gao, Ohlander, Jeppsson, Bjork, & Trajkovski, 2000).

2.5. Total phenol content

The amount of total phenolics of pepper extract was determined by the Folin-Ciocalteau method (Gao et al., 2000). Briefly, the extract was mixed with 0.2 ml Folin-Ciocalteau reagent, 2 ml of distilled water and 1 ml of 15% Na_2CO_3. The absorbance was measured at $\lambda = 765$ nm using a UV–vis Jenway 6003 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 dw.

2.6. HPLC flavonoids quantification

HPLC flavonoids (quercetin, kaempferol, luteolin) quantification was performed using following method reported by Wach, Pyrzyska, and Biesaga (2007). Pepper extracts were hydrolyzed with 2.8 M HCl in 60% MeOH at 90 °C for 10 min. The residue was treated with ethyl acetate. Then, the organic layer was dried and dissolved in 40% MeOH for analysis. Flavonoids content was calculated from the integrated peak area of the pepper sample and the corresponding standard. HPLC analyses were realized using an HPLC system HP 1100 equipped with a pump, UV–vis detector (280 nm), column oven, injector and a C18 RP column (Phenomenex Luna 5 μm C18, 250 × 4.60 mm). The mobile phase was H_2O/formic acid (0.1%) (A) and methanol (B) with a flow rate of 1 ml/min (2 min 100% A; 8 min 80% A; 55 min 100% B; 65 min 100% A).

2.7. Antioxidant activity

2.7.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

This experimental procedure was described by Blois (1958). In an ethanol solution of DPPH radical (final concentration of 1.0×10^{-4} M) extracts at different concentrations were added. The reaction mixture was shaken vigorously and then kept in the dark for 30 min. The absorbance of the resulting solutions was measured in 1 cm cuvettes using a UV–vis Jenway 6003 spectrophotometer at $\lambda = 517$ nm

against blank without DPPH. A decrease in the absorbance of the DPPH solution indicates an increase of DPPH radical scavenging activity.

2.7.2. ABTS test

ABTS assay was based on the method of Re et al. (1996) with slight modifications. ABTS radical cation ($ABTS^+$) was produced by the reaction of a 7 mM ABTS solution with 2.45 mM potassium persulphate. The mixture was stored in the dark at room temperature for 12 h before use. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.05 at $\lambda = 734$ nm. After addition of 25 μl of pepper extract or Trolox standard to 2 ml of diluted $ABTS^+$ solution, absorbance was measured at exactly 6 min after mixing. Appropriate solvent blanks were run in each assay. The scavenging ability of sample was calculated according to the following equation:

$$\text{ABTS scavenging activity}\,(\%) = [(A_0 - A)/A_0]\ \text{x}\ 100$$

where A_0 is the absorbance of the control reaction and A is the absorbance in the presence of sample. The IC_{50} value for each pepper extract, defined as the concentration of extract causing 50% inhibition of absorbance, was determined from the curves plotted and tabulated.

2.7.3. β-carotene bleaching test

Antioxidant activity was determined using the β-carotene bleaching test with some modifications (Menichini et al., 2009). Briefly, 1 ml of β-carotene solution (0.2 mg/ml in chloroform) was added to 0.02 ml of linoleic acid and 0.2 ml of 100% Tween 20. After evaporation of chloroform and dilution with water (100 ml), 5 ml of the emulsion were transferred into different test tubes containing 0.2 ml of samples in 70% ethanol at different concentrations. Standard (propyl gallate) at the same concentration as samples was used for comparison. The tubes were then gently shaken and placed at 45 °C in a water bath for 60 min. The absorbance of samples, standard and control was measured at $\lambda = 470$ nm against a blank, consisting of an emulsion without β-carotene. The measurement was carried out at initial time ($t = 0$) and successively at 30 and 60 min. All samples were assayed in triplicate and the mean value calculated.

2.8. Carbohydrate-hydrolyzing enzymes inhibitory activity

2.8.1. α-Amylase inhibitory activity

The α-amylase inhibition assay method was performed using the method previously described (Tundis et al., 2007). Briefly, a starch solution (0.5% w/v) was obtained by stirring 0.125 g of potato starch in 25 ml of 20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 65 °C for 15 min. The α-amylase (EC 3.2.1.1) solution was prepared by mixing 0.0253 g of α-amylase in 100 ml of cold distilled water. Samples were dissolved in buffer to give final concentrations ranging from 12.50 μg/ml to 1 mg/ml. The colorimetric reagent was prepared mixing a sodium potassium tartrate solution (12.0 g of sodium potassium tartrate, tetrahydrate in 8.0 ml of 2 M NaOH) and 96 mM 3,5-dinitrosalicylic acid solution. Control, total extracts and lipophilic fractions were added to starch solution and left to react with α-amylase solution at 25 °C for 5 min. The reaction was measured over 3 min. The generation of maltose was quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid, the product being detectable at $\lambda = 540$ nm. The IC_{50} value for each pepper extract, defined as the concentration of extract causing 50% enzyme inhibition was determined from the curves plotted and tabulated. The absolute inhibition constant K_i was determined following the Cheng-Prusoff equation (Leff & Dougall, 1993).

2.8.2. α-Glucosidase inhibitory activity

The α-glucosidase inhibition was measured through a modified Sigma-Aldrich bioassay method (Loizzo et al., 2008). A maltose solution (4% w/v) was prepared by dissolving 12 g of maltose in 300 ml of 50 mM sodium acetate buffer. The enzyme solution was prepared by mixing 1 mg of α-glucosidase (10 units/mg) in 10 ml of ice-cold distilled water. Samples were dissolved in the buffer to give a final concentration ranging from 5 μg/ml to 1 mg/ml. The colorimetric reagent *o*-dianisidine (DIAN) solution was prepared by dissolving 1 tablet in 25 ml of distilled water, while the peroxidase/glucose oxidase (PGO) system-colour reagent solution was prepared fresh by dissolving 1 capsule in 100 ml of ice-cold distilled water. In the first step both control and samples were added to maltose solution and left to equilibrate at 37 °C. The reaction was started by adding α-glucosidase solution and tubes were left to incubate at 37 °C for 30 min. After that time perchloric acid solution (4.2% w/v) was added to stop reaction. In the second step the generation of glucose was quantified by the reduction of DIAN. The supernatant of tube of step I was mixed with DIAN and PGO and was left to incubate at 37 °C for 30 min. The absorbance of DIAN was measured spectrophotometrically at $\lambda = 500$ nm. The IC_{50} value for each pepper extract, defined as the concentration of extract causing 50% enzyme inhibition was determined from the curves plotted and tabulated. K_i value was determined following the Cheng-Prusoff equation (Leff & Dougall, 1993).

2.9. Statistical analysis

All experiments were carried out in triplicate. Data were expressed as means ± standard deviation (S.D.). The concentration giving 50% inhibition (IC_{50}) was calculated by nonlinear regression with the use of Prism Graphpad version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). The dose–response curve was obtained by plotting the percentage inhibition *versus* concentration. Differences within and between groups were evaluated by one-way analysis of variance test (ANOVA) followed by a multicomparison Dunnett's test compared with the positive controls.

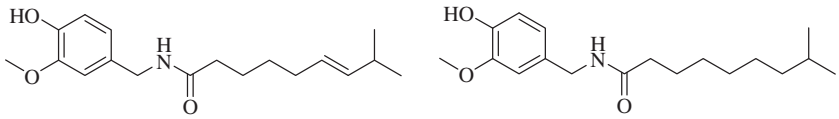
3. Results and discussion

3.1. Phytochemical results

The two major capsaicinoids in pepper fruits are capsaicin and dihydrocapsaicin (Fig. 1). These capsaicinoids are responsible for up to 90% of the total characteristic pungent taste. In addition to sensory properties, capsaicinoids are reported to possess beneficial biological properties that may affect human health (Krzyzanowska, Czubacka, & Oleszek, 2011). They have also exhibited protective effects against mutagens and carcinogens, cholesterol, and obesity (Kakigi, Inui, & Tamura, 2005; Kempaiah, Manjunatha, & Srinivasan, 2005; Laviada, 2006; Westerterp-Plantenga, Smeets, & Lejeune, 2005). *C. annuum* var. *acuminatum* medium was characterized by highest levels of capsaicin and dihydrocapsaicin with values of 1.4 and 0.5 mg/g, respectively (Table 1).

The GC-MS analysis of the lipophilic fraction of both pepper cultivars was as in Table 2. Both *C. annuum* var. *acuminatum* cultivars showed fatty acids, methyl and ethyl esters, and sterols as major components. The most abundant constituents identified in the *n*-hexane fraction of *C. annuum* var. *acuminatum* medium were methyl palmitate (12.32%), linoleic acid (11.50%), palmitic acid (11.26%), 14-methyl-palmitic acid methyl ester (4.89%), phytol (4.84%), and vitamin E (4.00%). *C. annuum* var. *acuminatum* big showed a major content in palmitic acid (13.04%), methyl palmitate (10.14%), linoleic acid (9.57%) and ethyl linolenate (4.77%). This cultivar possessed the highest content of phytol (4.99%), vitamin E (5.53%), and ethyl palmitate (3.02%). On the contrary, *C. annuum* var. *acuminatum* medium showed a major content of myristic acid (2.68%), methyl linoleate (2.34%) and methyl linolenate (1.74%).

Peppers are also a good source of carotenoids, which can vary in composition and concentration owing to differences in genetics and maturation (Markus, Daood, Kapitany, & Biacs, 1999). All the carotenoid pigments present in the pepper are C40 isoprenoids containing nine



Capsaicin **Dihydroapsaicin**

Fig. 1. Two main capsaicinoids in pepper.

conjugated double bonds in the central polyenic chain, although with different end groups, which change the chromophore properties of each pigment, allowing them to be classified into two isochromic families: red and yellow. The red fraction contains the pigments exclusive to the *Capsicum* genus (capsanthin, capsanthin 5,6-epoxide, and capsorubin), and the yellow fraction comprises the rest of the pigments (zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, β-carotene and cucurbitaxanthin A). Ha, Kim, Park, Lee, and Cho (2007) analyzed the accumulation of carotenoids in *Capsicum* fruits at fully ripen maturity stage concluding that capsanthin was found to be the major component (80%) of the total carotenoids in the ripe fruits of red peppers. The levels of other β-carotenoids including β-carotene, β-cryptoxanthin, and zeaxanthin varied from 1.5% to 16%, and that of lutein was never detected above 0.3% of the total carotenoid content in most of the red peppers. The total carotenoid content was determined by measuring the absorption of lipophilic fractions at 460 nm. A good carotenoids content was evidenced for *C. annuum* var. *acuminatum* medium lipophilic fraction with a value of 324.7 mg/100 g of plant materials. Suzuki, Mayumi, Ishikawa, Takizawa, and Nunomura (2007) reported that normally significant positive correlations were observed between β-carotene and capsanthin contents. Based on these observations, irrespective of the total quantity of carotenoids and their relative proportion of capsanthin in *C. annuum* fruit, it is assumed that the ratio of β-carotene to capsanthin is constant and the ratio of 1:10 could thus potentially be used as an index to analyze the content of these carotenoids. In our previous work, we have analyzed the carotenoid content of *C. annuum* var. *acuminatum* small lipophilic fraction founding a very close value of 324.2 mg/100 g of plant materials (Tundis et al., 2011).

Moreover, Collera-Zúñiga, Garcìa-Jiménez, and Meléndez-Gordillo (2005) analyzed the carotenoid composition of three *C. annuum* varieties founding carotenoid content of 6.76 mg/100 g dw for Guajillo and a range of 7.0–7.5 mg/100 g dw for Ancho and Mulato varieties. This content is considerably low in comparison to our results.

Pepper fruits contain a wide array of phytochemicals with well-known antioxidant properties including polyphenols. Pepper extracts were analyzed in order to evaluate the total phenol content by the Folin-Ciocalteau method. As shown in Table 1, both *C. annuum* var. *acuminatum* medium and big were characterized by high content of polyphenols (843.7 and 748.7 mg/100 g dw for medium and big, respectively). A similar polyphenols content was found in *C. chinense* Habanero (759.12 mg/100 g fw) (Menichini et al., 2009). An high polyphenols content was found in *C. annuum* var. *acuminatum* small (970.2 mg/100 g dw) (Tundis et al., 2011) while Kevers et al. (2007) found in red, yellow and green fresh peppers a lower polyphenolic content with values of 296, 284 and 215 mg/100 g fw, respectively.

Table 1
Total phenols, carotenoids, capsaicin and dihydrocapsaicin content in *C. annuum* var. *acuminatum* medium and big.

Phytochemicals	*C. annuum* var. *acuminatum* medium	*C. annuum* var. *acuminatum* big
Phenols (mg/100 g dw)	843.7 ± 2.6	748.7 ± 3.7
Carotenoids (mg/100 g dw)	324.7 ± 1.3	191.7 ± 1.0
Capsaicin (mg/g dw)	1.4 ± 0.01	0.9 ± 0.02
Dihydrocapsaicin (mg/g dw)	0.5 ± 0.03	4.7 ± 0.02

Data represents the mean ± standard deviation S.D. ($n = 3$); dw: dried weight.

Following method previously published by Howard et al. (2000) and Kim et al. (2011) applied to pepper, we have decided to quantify quercetin, luteolin and kaempferol, the most common aglycones recognized in pepper fruits, by HPLC (Table 3) considering that quantitative determination of individual flavonoid glycosides in plant materials is difficult, due to their large number.

Interestingly, *C. annuum* var. *acuminatum* big is characterized by the highest quercetin, luteolin and kaempferol with 68.0, 87.6 and 29.7 μg/g dw, respectively. The quercetin and luteolin content values are 10 times higher than *C. annuum* var. *acuminatum* medium. These results are in agreement with previous studies that analyzed different pepper cultivars. In our investigation on *C. annuum* var. *acuminatum* small quercetin resulted the most high flavonoid contained with value of 56 μg/g dw followed by luteolin and kaempferol with 9.7 and 8.7 μg/g dw, respectively (Tundis et al., 2011).

Table 2
Major non-polar components of *C. annuum* var. *acuminatum* medium (M) and *C. annuum* var. *acuminatum* big (B) *n*-hexane fractions analyzed by GC-MS.

I[a]	Compound	(M)[b]	(B)[b]	Identification[c]
1500	Pentadecane	0.57 ± 0.08	0.49 ± 0.06	A
1614	Tetradecanal	0.62 ± 0.05	0.29 ± 0.05	B
1700	Heptadecane	1.28 ± 0.01	1.57 ± 0.06	B
1707	Pentadecanal	0.95 ± 0.01	1.10 ± 0.01	B
1811	Hexadecanal	1.38 ± 0.03	1.80 ± 0.03	B
1830	Neophytadiene	1.03 ± 0.03	1.47 ± 0.01	B
1863	Methyl pentadecanoate	2.66 ± 0.09	2.58 ± 0.08	B
1883	Pentadecanoic acid	1.85 ± 0.07	1.10 ± 0.07	B
1934	Methyl palmitate	12.32 ± 0.11	10.14 ± 0.16	A
1950	Phytol	4.84 ± 0.09	4.99 ± 0.03	B
1955	14-Methyl-palmitic acid methyl ester	4.89 ± 0.03	3.85 ± 0.05	B
1969	Palmitic acid	11.26 ± 0.24	13.04 ± 0.21	A
1975	Ethyl palmitoleate	3.85 ± 0.03	5.12 ± 0.06	B
1987	Myristic acid	2.68 ± 0.11	1.86 ± 0.03	B
1996	Methyl linoleate	2.34 ± 0.03	1.43 ± 0.05	B
1999	Methyl linolenate	1.74 ± 0.05	0.82 ± 0.05	B
2000	Eicosane	0.68 ± 0.04	0.85 ± 0.04	A
2005	Ethyl palmitate	2.32 ± 0.01	3.02 ± 0.03	A
2024	Octadecanal	0.21 ± 0.03	0.25 ± 0.07	B
2133	Methyl stearate	2.51 ± 0.01	2.79 ± 0.08	B
2156	Linoleic acid	11.50 ± 0.11	9.57 ± 0.12	A
2160	2-Monolinolenin	2.04 ± 0.01	0.94 ± 0.01	B
2167	Ethyl linolenate	3.52 ± 0.07	4.77 ± 0.09	B
2186	Methyl arachidate	1.61 ± 0.10	1.92 ± 0.08	B
2197	Ethyl stearate	1.75 ± 0.06	1.71 ± 0.09	B
2292	5-Eicosene	0.69 ± 0.04	0.53 ± 0.01	B
	Methyl behenate	0.92 ± 0.03	0.33 ± 0.01	B
2500	Pentacosane	0.41 ± 0.02	1.92 ± 0.02	B
2600	Hexacosane	0.46 ± 0.01	1.81 ± 0.03	B
2700	Heptacosane	1.43 ± 0.12	1.77 ± 0.08	B
2900	Nonacosane	1.27 ± 0.03	1.76 ± 0.11	B
	Vitamin E	4.00 ± 0.18	5.53 ± 0.24	A
	Campesterol	0.82 ± 0.01	0.67 ± 0.02	C
	Stigmasterol	2.21 ± 0.07	1.94 ± 0.12	C
	Stigmast-6-en-3-ol	1.62 ± 0.08	1.21 ± 0.06	C
	β-Sitosterol	0.87 ± 0.01	0.76 ± 0.04	A
	β-Amyrin	0.54 ± 0.09	0.59 ± 0.06	C

[a] *I*, Retention Index on MS HP-5 non polar column. [b] Abundance calculated as % peak area mean values, mean ± standard deviation ($n = 3$). [c] The reliability of the identification proposal is indicated by the following: A, mass spectrum and retention index agreed with standards; B, mass spectrum and retention index agreed with database or literature; C, mass spectrum agreed with mass spectral database.

Table 3
Quantitative analysis by HPLC of flavonoids *C. annuum* var. *acuminatum* medium and big.

	Flavonoids[a]		
	Quercetin	Luteolin	Kaempferol
C. annuum var. *acuminatum* medium	5.6 ± 0.6	8.9 ± 0.8	6.8 ± 0.5
C. annuum var. *acuminatum* big	68.0 ± 1.4	87.6 ± 1.9	29.7 ± 1.3

Data represents the mean ± standard deviation S.D. ($n = 3$). [a] μg/g dried weight (dw).

Ghasemnezhad, Sherafati, and Payvast (2011) reported the content of quercetin in *C. annuum* in five different pepper genotypes, Arian, Marona, Zorro, Y-43-09 and Y-43-07 that ranging from 37.63 to 117.58 μg/g dw while *C. annuum* var. *special* presented a quercetin value of 4.76 and 3.29 μg/g dw for green and red pepper, respectively (Kim et al., 2011). Both peppers are characterized by a lower value of luteolin (0.64 and 0.36 μg/g dw for green and red pepper, respectively).

3.2. Antioxidant activity

Considering that different antioxidant compounds may act *in vivo* through different mechanisms of action, no single method can fully evaluate the antioxidant capacity of food since levels of single antioxidant in food do not necessarily reflect their antioxidant activity (Pellegrini et al., 2003). Therefore, to investigate the antioxidant activity choosing an adequate assay is critical. For this reason *C. annuum* var. *acuminatum* medium and big extracts were evaluated for their radical scavenging activity through DPPH and ABTS assay and for their antioxidant potential through β-carotene bleaching test. Data are reported in Table 4. All extracts were able to reduce the stable free radical DPPH˙ to the yellow-colored DPPH (Fig. 2). The best free radical scavenging activity was exerted by *C. annuum* var. *acuminatum* medium with an IC_{50} value of 85.3 μg/ml. A lower radical scavenging activity was obtained also with *C. annuum* var. *acuminatum* small and *C. annuum* var. *cerasiferum* (IC_{50} values of 152.9 and 463.0 μg/ml, respectively) (Tundis et al., 2011). The influence of stage of ripeness on the radical scavenging activity of *C. annuum* var. *acuminatum* was investigated by Conforti, Statti, and Menichini (2007). At full maturity stage peppers showed a modest radical scavenging activity with an IC_{50} value of 419.0 μg/ml.

Moreover, *C. annuum* var. *acuminatum* big was most active than *C. chinense* Habanero that exhibited an IC_{50} value of 287 μg/ml (Menichini et al., 2009) and also than *C. annuum* var. *special* that presented IC_{50} values of 286.23 and 150.40 μg/ml for green and red pepper, respectively (Kim et al., 2011). Conversely, *C. annuum* var. *acuminatum* big showed an interesting radical scavenging activity in ABTS assay (IC_{50} value of 16.4 μg/ml) and the highest inhibition of linoleic acid oxidation (IC_{50} values of 1.2 μg/ml and 2.9 μg/ml at 30 min and 60 min of incubation, respectively) (Fig. 3).These results are important if compared to propyl gallate used as positive control (IC_{50} value of 1.0 μg/ml at 30 min of incubation). Similar results were obtained with *C. chinense* Habanero that showed a significant activity in mature stage (IC_{50} value of 4.6 μg/ml after 30 min of incubation) (Menichini et al., 2009) and *C. annuum* var. *cerasiferum* (IC_{50} value of 3.1 μg/ml after 30 min of incubation) (Tundis et al., 2011). A lower bioactivity was observed with *C. annuum* var. *acuminatum* small that showed an IC_{50} value of 16.3 μg/ml at 30 min of incubation. Anyway the antioxidant activity of the extracts decreased during the reaction time (Tundis et al., 2011). These considerable results in the inhibition the decoloration of β-carotene may be correlated with the high content in carotenoids and capsaicinoids. The major pungent components in *Capsicum* plants, capsaicin and dihydrocapsaicin, can inhibit iron-mediated lipid peroxidation and copper-dependent oxidation of low-density lipoprotein, an effect ascribed to their capacity to form complexes with reduced metals and act as hydrogen donors (Rosa et al., 2002). Capsaicin could also prevent the oxidation of oleic acid at cooking temperatures as well as the formation of lipid hydroperoxides from the autoxidation of linoleic acid. Also carotenoids have been recently and widely studied and have been proven to play an important role in preventing oxidative damage which is caused by free radicals in age-related diseases. Most current research is focused on a proposed role of carotenoids as lipid antioxidants which can protect against oxidation and other destructive processes mediated by singlet oxygen and free radicals, though more specific effects on the immune system are now under investigation (Tapiero, Townsend, & Tew, 2004). The same role could be attributed to phenolic compounds (Tabart, Kevers, Pincemail, Defraigne, & Dommes, 2009).

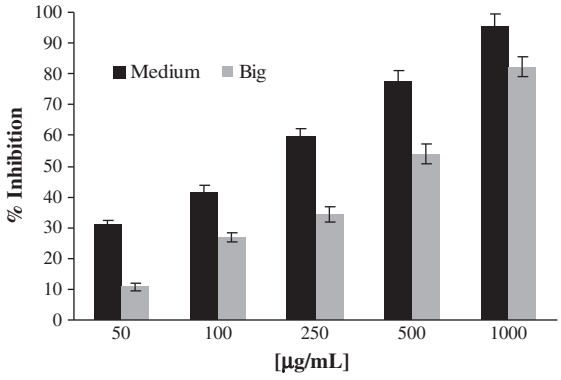


Fig. 2. Radical scavenging activity using DPPH assay of *C. annuum* var. *acuminatum* medium and big fruits. Data are mean ± S.D. ($n = 3$).

3.3. Carbohydrate-hydrolyzing enzymes inhibitory activity

α-Amylase and α-glucosidase inhibitors are currently used to reduce glucose post-prandial plasma level in diabetes type 2 and in case of obesity. In our continuous investigation on pepper biological properties we have investigated the *C. annuum* var. *acuminatum* medium and big total extracts and lipophilic fractions for their carbohydrate-hydrolyzing enzymes inhibitory activities. A concentration-response relationship was observed (Fig. 4a). The IC_{50} and Ki values were summarized in Table 5. The total extract of both cultivars are able to inhibit α-glucosidase with IC_{50} values of 73.4 μg/ml ($Ki = 1.3 \times 10^{-4}$) and

Table 4
Radical scavenging and antioxidant capacities of *C. annuum* var. *acuminatum* medium and big extracts.

Sample	DPPH[a]	ABTS[a]	β-Carotene bleaching test[a]	
			30 min	60 min
C. annuum var. *acuminatum* medium	85.3 ± 0.029**	21.5 ± 0.08 **	12.7 ± 0.012**	15.2 ± 0.012**
C. annuum var. *acuminatum* big	137.9 ± 0.015**	16.4 ± 0.04**	1.2 ± 0.005**	2.9 ± 0.010**
Propyl gallate[a]	–	–	1.0 ± 0.01	1.0 ± 0.01
Ascorbic acid[a]	2.0 ± 0.01	1.72 ± 0.03	–	–

Data represents the mean ± standard deviation S.D. ($n = 3$); [a]IC_{50} μg/ml; DPPH Radical Scavenging Activity Assay; Antioxidant Capacity Determined by Radical Cation ($ABTS^{+}$), β-Carotene bleaching test; [a] Propyl gallate and ascorbic acid were used as positive control. Differences within and between groups were evaluated by one-way analysis of variance test *** $P < 0.0001$ followed by a multicomparison Dunnett's test: ** $P < 0.01$ compared with the positive controls.

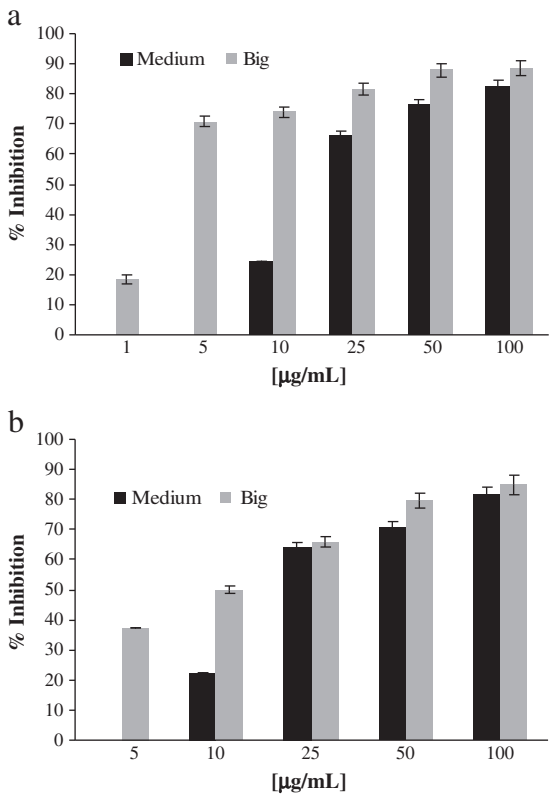


Fig. 3. Lipid peroxidation inhibition using the β-carotene-linoleic acid system after a) 30 min of incubation and b) 60 min of incubation of *C. annuum* var. *acuminatum* medium and big. Data are mean ± S.D. ($n = 3$).

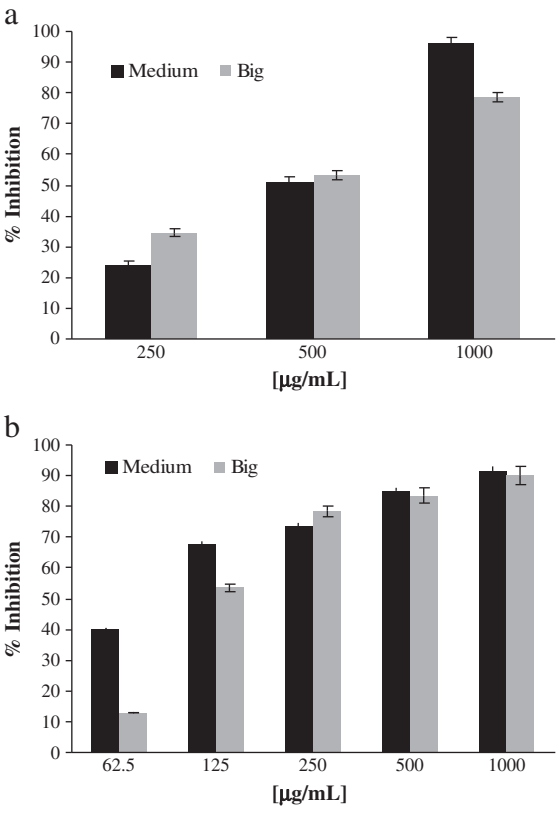


Fig. 4. *C. annuum* var. *acuminatum* medium and big inhibitory activity against a) α-amylase and b) α-glucosidase. Data are mean ± S.D. ($n = 3$).

119.2 μg/ml ($Ki = 3.5 \times 10^{-4}$) for medium and big, respectively (Fig. 4b). A lower bioactivity was evidence against α-amylase. The total extract from *C. annuum* var. *acuminatum* medium was characterized by the highest phenols content. According to Mai, Thu, Tien, and Chuyen (2007) positive relationships among α-glucosidase inhibitory activity, radical scavenging activity and total phenols content were found. Li, Zhou, Gao, Bian, and Shan (2009) analyzed the inhibitory activity of quercetin against α-glucosidase founding an IC_{50} value of 0.017 mmol/l. A lower activity was observed with luteolin that inhibited the enzyme with a percentage of 36% at concentration of 0.5 mg/ml (Kim, Kwon, & Son, 2000). Previously, we have investigated the *in vitro* hypoglycemic activity of fresh *C. annuum* var. *acuminatum* fruits at different ripening stages. The mature red fruits inhibited selectively α-glucosidase with an IC_{50} value of 143.71 μg/ml (Loizzo et al., 2008). A different effect was observed with another *Capsicum* species. The mature *C. chinense* Habanero total extract exhibited an IC_{50} value of 131 μg/ml against α-amylase while a lower activity was found for α-glucosidase with an IC_{50} value of 265 μg/ml (Menichini et al., 2009). Recently, Oboh et al. (2011) reported the ability of combination of the three *C. annuum* varieties (1:1:1) including *C. annuum* var. *grossum*, *C. annuum* var. *abbreviatum*, and *C. annuum* var. *accuminatum* to inhibit carbohydrate-hydrolyzing enzymes. The combination of peppers showed additive in the inhibition of pancreatic α-amylase whereas α-glucosidase inhibitory activity was antagonistic with the combination. *C. annuum* var. *accuminatum* contributed most to the activities of the combined peppers.

In order to evaluate the compounds responsible for the activity, the lipophilic fraction was also tested. Unlike to the total extracts lipophilic fractions exhibited a selective activity against α-amylase with IC_{50} values of 8.7 and 29.0 μg/ml for big and medium pepper, respectively. Both fractions have an interesting content in linoleic acid (LA). LA is an unsaturated ω-6 fatty acid and is the most abundant polyunsaturated fatty acid in the diet. A series of studies have shown that diabetic patients require higher than normal intakes of LA. Because they have consistently been shown to have above normal levels of LA while having lower than normal levels of γ-linolenic acid, it is believed that diabetics have impaired Δ-6-desaturase activity. Increased intakes of LA have been shown to attenuate diabetic complications and improve insulin sensitivity (Davì, Santilli, & Patrono, 2010; Summers et al., 2002). The evidence that pepper consumption might have clinical implications in the management of type 2 diabetes was confirmed also by *in vivo* study. In fact, Chaiyasit et al. (2009) reported the crossover study performed in 12 healthy volunteers that receiving 5 grams of *C. frutescens* each day. After measurement of Oral Glucose Tolerance Test (OGTT), insulin and capsaicin level in plasma, authors concluded that pepper consumption contains capsaicin levels that were associated with a decrease in plasma glucose levels and the maintenance of insulin levels *in vivo*.

4. Conclusion

In conclusion, studies on health benefits of edible plant and spice bioactive constituents have attracted the interest of scientists seeking to prevent disease and promote health. In this context the present study evaluated the antioxidant and hypoglycaemic properties of two *C. annuum* var. *acuminatum* cultivars (medium and big), widely used in the traditional Mediterranean diet. The total phenols, carotenoids and capsaicinoids content and the HPLC flavonoids quantification were investigated. Particularly important are the results of antioxidant activity through different *in vitro* systems. Furthermore, peppers showed appreciable inhibitory activity against the carbohydrate-hydrolyzing enzymes α-amylase and α-glucosidase. Although pepper finds wide application as a flavoring and preservative with this work we would valorize this spice for its nutraceutical value and as therapeutic agent in the treatment and prevention of human diseases.

Table 5
Hypoglycaemic activity of total extracts and lipophilic fractions of *C. annuum* cultivars.

Sample	α-amylase		α-glucosidase	
	IC_{50} (μg/ml)	Ki	IC_{50} (μg/ml)	Ki
Total extract				
C. annuum var. *acuminatum* medium	486.8 ± 3.5**	0.04	73.4 ± 1.1**	1.3×10^{-4}
C. annuum var. *acuminatum* big	334.9 ± 3.1**	0.02	119.2 ± 2.1**	3.5×10^{-4}
Lipophilic fraction				
C. annuum var. *acuminatum* medium	29.0 ± 1.1**	1.7×10^{-4}	>1000	–
C. annuum var. *acuminatum* big	8.7 ± 0.7**	8.7×10^{-3}	>1000	–
Acarbose	50.0 ± 0.9	45.45	35.5 ± 1.2	16.7

Data represents the mean ± standard deviation S.D. (n = 3). Differences within and between groups were evaluated by one-way analysis of variance (ANOVA) test completed with a multicomparison Dunnett's test. **$p<0.01$ compared with the positive control (Acarbose).

Acknowledgement

The authors are grateful to Miceli Farm s.r.l., Italy, for supplying the pepper fruits.

References

Ahuja, K. D., Robertson, I. K., Geraghty, D. P., & Ball, M. J. (2006). Effects of chili consumption on postprandial glucose, insulin, and energy metabolism. *American Journal of Clinical Nutrition*, *84*(1), 63–69.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199–1200.

Chaiyasit, K., Khovidhunkit, W., & Wittayalertpanya, S. (2009). Pharmacokinetic and the effect of capsaicin in *Capsicum frutescens* on decreasing plasma glucose level. *Journal of the Medical Association of Thailand*, *92*(1), 108–113.

Collera-Zúñiga, O., Garcìa-Jiménez, F., & Meléndez-Gordillo, R. (2005). Comparative study of carotenoid composition in three Mexican varieties of *Capsicum annuum* L. *Food Chemistry*, *90*(1–2), 109–114.

Conforti, F., Statti, G. A., & Menichini, F. (2007). Chemical and biological variability of hot pepper fruits (*Capsicum annuum* var. *acuminatum* L.) in relation to maturity stage. *Food Chemistry*, *102*(4), 1096–1104.

Davì, G., Santilli, F., & Patrono, C. (2010). Nutraceuticals in diabetes and metabolic syndrome. *Cardiovascular Therapy*, *28*(4), 216–226.

Fowles, J., Mitchell, J., & McGrath, H. (2001). Assessment of cancer risk from ethylene oxide residues in spices imported into New Zealand. *Food and Chemical Toxicology*, *39*(11), 1055–1062.

Gao, X., Ohlander, M., Jeppsson, N., Bjork, L., & Trajkovski, V. (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of Sea buckthorn (*Hippophae rhamnoides* L.) during maturation. *Journal of Agricultural and Food Chemistry*, *48*(5), 1485–1490.

Ghasemnezhad, M., Sherafati, M., & Payvast, G. A. (2011). Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times. *Journal of Functional Foods*, *3*(1), 44–49.

Ha, S. H., Kim, J. B., Park, J. S., Lee, S. W., & Cho, K. J. (2007). A comparison of the carotenoid accumulation in Capsicum varieties that show different ripening colours: deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. *Journal of Experimental Botany*, *58*(12), 3135–3144.

Howard, L. R., Talcott, S. T., Brenes, C. H., & Villalon, B. (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *Journal of Agricultural and Food Chemistry*, *48*(5), 1713–1720.

Kakigi, R., Inui, K., & Tamura, Y. (2005). Electrophysiological studies on human pain perception. *Clinical Neurophysiology*, *116*(4), 743–763.

Kempaiah, R. K., Manjunatha, H., & Srinivasan, K. (2005). Protective effect of dietary capsaicin on induced oxidation of low-density lipoprotein in rats. *Molecular and Cellular Biochemistry*, *275*(1–2), 7–13.

Kevers, C., Falkowski, M., Tabart, J., Defraigne, J. O., Dommes, J., & Pincemail, J. (2007). Evolution of antioxidant capacity during storage of selected fruits and vegetables. *Journal of Agricultural and Food Chemistry*, *55*(21), 8596–8603.

Kim, J. S., Ahn, J., Lee, S. J., Moon, B. K., Ha, T. Y., & Kim, S. (2011). Phytochemicals and antioxidant activity of fruits and leaves of paprika (*Capsicum annuum* L., var. special) cultivated in Korea. *Journal of Food Science*, *76*(2), C193–C198.

Kim, J. S., Kwon, C. S., & Son, K. H. (2000). Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Bioscience, Biotechnology, and Biochemistry*, *64*(11), 2458–2461.

Krzyzanowska, J., Czubacka, A., & Oleszek, W. (2011). Dietary phytochemicals and human health. *Advances in Experimental Medicine and Biology*, *698*, 74–98.

Laviada, I. (2006). Induction of apoptosis in prostate tumor PC-3 cells and inhibition of xenograft prostate tumor growth by the vanilloid capsaicin. *Apoptosis*, *11*(1), 89–99.

Lee, Y., Howard, L. R., & Villalon, B. (1995). Flavonoids and antioxidant activity of fresh pepper (*Capsicum annuum*) cultivars. *Journal of Food Science*, *60*(3), 473–476.

Leff, P., & Dougall, I. G. (1993). Further concerns over Cheng-Prusoff analysis. *Trends in Pharmacological Sciences*, *14*(4), 110–112.

Li, Y. Q., Zhou, F. C., Gao, F., Bian, J. S., & Shan, F. (2009). Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of alpha-glucosidase. *Journal of Agricultural and Food Chemistry*, *57*(24), 11463–11468.

Loizzo, M. R., Tundis, R., Menichini, F., Statti, G. A., & Menichini, F. (2008). Influence of ripening stage on health benefits properties of *Capsicum annuum* var. *acuminatum* L.: *in vitro* studies. *Journal of Medicinal Food*, *11*(1), 184–189.

Mai, T. T., Thu, N. N., Tien, P. G., & Chuyen, N. V. (2007). Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. *Journal of Nutritional Science and Vitaminology*, *53*(3), 267–276.

Markus, F., Daood, H. G., Kapitany, J., & Biacs, P. A. (1999). Change in the carotenoid and antioxidant content of spice red pepper (paprika) as a function of ripening and some technological factors. *Journal of Agricultural and Food Chemistry*, *47*(1), 100–107.

Matsufuji, H., Nakamura, H., Chino, M., & Takeda, M. (1998). Antioxidant activity of capsanthin and the fatty acid esters in paprika (*Capsicum annuum*). *Journal of Agricultural and Food Chemistry*, *46*(9), 3468–3472.

Menichini, F., Tundis, R., Bonesi, M., Loizzo, M. R., Conforti, F., Statti, G., et al. (2009). The influence of fruit ripening on the phytochemical content and biological activity of *Capsicum chinense* Jacq. cv Habanero. *Food Chemistry*, *114*(2), 553–560.

Nicasio-Torres, M. P., Erazo-Gómez, J. C., & Cruz-Sosa, F. (2009). *In vitro* propagation of two antidiabetic species known as guarumbo: *Cecropia obtusifolia* and *Cecropia peltata*. *Acta Physiologiae Plantarum*, *31*(5), 905–914.

Oboh, G., Ademiluyi, A. O., & Faloye, Y. M. (2011). Effect of combination on the antioxidant and inhibitory properties of tropical pepper varieties against α-amylase and α-alucosidase activities: *in vitro*. *Journal of Medicinal Food*. doi:10.1089/jmf.2010.0194.

Osuna-Garcia, J. A., Wall, M. M., & Waddell, C. A. (1998). Endogenous levels of tocopherols and ascorbic acid during fruit ripening of new Mexican-type Chile (*Capsicum annuum* L.) cultivars. *Journal of Agricultural and Food Chemistry*, *46*, 5093–5096.

Pellegrini, M., Serafini, B., Colombi, D., del Rio, S., Salvatora, M., & Bianchi Brighenti, F. (2003). Total antioxidant capacity of plant foods, beverages and oils consumed in Italy by three different *in vitro* assays. *Journal of Nutrition*, *133*(9), 2812–2819.

Ranilla, L. G., Kwon, Y. I., Apostolidis, E., & Shetty, K. (2010). Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresource Technology*, *101*(12), 4676–4689.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1996). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, *26*(9–10), 1231–1237.

Rosa, A., Deiana, M., Casu, V., Paccagnini, S., Appendino, G., Ballero, M., et al. (2002). Antioxidant activity of capsinoids. *Journal of Agricultural and Food Chemistry*, *50*(25), 7396–7401.

Summers, L. K. M., Fielding, B. A., Bradshaw, H. A., Ilic, V., Beysen, C., Clark, M. L., et al. (2002). Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. *Diabetologia*, *45*(3), 369–377.

Suzuki, K., Mayumi, M., Ishikawa, K., Takizawa, K., & Nunomura, O. (2007). Carotenoid Composition in Mature *Capsicum annuum*. *Food Science and Technology Research*, *13*(1), 77–80.

Tabart, J., Kevers, C., Pincemail, J., Defraigne, J. -O., & Dommes, J. (2009). Comparative antioxidant capacities of phenolic compounds measured by various tests. *Food Chemistry*, *113*(4), 226–1233.

Tapiero, H., Townsend, D. M., & Tew, K. D. (2004). The role of carotenoids in the prevention of human pathologies. *Biomedicine & Pharmacotherapy*, *58*(2), 100–110.

Tolan, I., Ragoobirsingh, D., & Morrison, E. Y. (2004). Isolation and purification of the hypoglycaemic principle present in *Capsicum frutescens*. *Phytotherapy Research*, *18*(1), 95–96.

Tundis, R., Loizzo, M. R., Statti, G. A., & Menichini, F. (2007). Inhibitory effects on the digestive enzyme α-amylase of three *Salsola* species (Chenopodiaceae) *in vitro*. *Pharmazie*, *62*(6), 473–475.

Tundis, R., Loizzo, M. R., Menichini, F., Bonesi, M., Conforti, F., Statti, G.,De Luca, D., de Cindio, B., & Menichini, F. (2011). Comparative study on the chemical composition, antioxidant properties and hypoglycaemic activities of two *Capsicum annuum* L. cultivars (acuminatum small and cerasiferum). *Plant Food for Human Nutrition*, *66*(3), 261-269.

Wach, A., Pyrzyska, K., & Biesaga, M. (2007). Quercetin content in some food and herbal samples. *Food Chemistry*, *100*(2), 699–704.

Westerterp-Plantenga, M. S., Smeets, A., & Lejeune, M. P. (2005). Sensory and gastrointestinal satiety effects of capsaicin on food intake. *International Journal of Obesity*, *29*(6), 682–688.

WHO (2009). *World Health and Organization.* Prevalence data of diabetes worldwide.