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EFFECTS OF SUN-DRYING ON THE ANTIOXIDANT POTENTIALS OF PEPPER (CAPSICUM) VARIETIES

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ABSTRACT

The present study investigated the effects of sun-drying on the antioxidant potential of three pepper varieties: *Capsicum annuum* var, *Capsicum chinense* and *Capsicum annuum*. Fresh fruits of the pepper varieties were collected, washed under distilled water and were divided into two parts: one for fresh sample and the other for the dried sample. Dried and fresh samples of the pepper varieties were homogenized and extracted with methanol. The concentrations of total phenolics and flavonoids were evaluated; DPPH-radical scavenging activity and the FRAP potential of the extracts were also determined. The results revealed that sun-drying process significantly reduced the total phenolic content of *C. annuum* var, *C. chinense* and *C. annuum* from 5.91 ± 0.22 mg/g GAE, 6.9 ± 0.23 mg/g GAE, 6.67 ± 0.99 mg/g GAE to 3.31 ± 0.72 mg/g GAE, 3.59 ± 0.89 mg/g GAE, 3.01 ± 0.17 mg/g GAE respectively and flavonoid content from 3.80 ± 0.02 mg/g QE, 3.91 + 0.08 mg/g QE, 3.84 ± 0.08 mg/g QE to 1.26 ± 0.90 mg/g QE, 1.95 ± 0.07 mg/g QE, 1.23 ± 0.04 mg/g QE respectively. The result also revealed that the fresh samples of *C. annuum* var, *C. chinense* and *C. annuum* exhibited higher percentage inhibition of DPPH-radical at 59.4 ± 0.5%, 61.2 ± 0.6%, 58.9 ± 0.2% respectively and were significantly different from the percentage inhibition by the dried samples: 39.2 ± 0.5%, 42.4 ± 0.4%, 38.6 ± 0.6% respectively.The FRAP potential of the fresh samples of *C. annuum* var, *C. chinense* and *C. annuum*: 588.56 ± 29.4 ìmol Fe(II)/g, 691.34 ± 20.46 ìmol Fe(II)/g and 598.9 ± 23. 82 ìmol Fe(II)/g respectively were significantly different from the dried samples: 370.22 ± 14.75 ìmol Fe(II)/g, 392.34 ± 45.74 ìmol Fe(II)/g and 358.6 ± 30.08 ìmol Fe(II)/g respectively. The three *Capsicum* species are very rich in antioxidants. However, the sun drying method reduced the antioxidant capacities of the peppers, thus further studies should be carried out on the best method for the preservation of *Capsicum* species.

Key Words: *Capsicum. annuum* var*, C. chinense*, *C. annuum*, Antioxidant, Sun-drying, methanolic extract

INTRODUCTION

For centuries, plants have been successfully used for the benefits of human health (Andrews, 1999). The interest in the consumption of pepper fruits (*Capsicum*) to a large extent is due to its phytochemical content and their importance as dietary antioxidants. Peppers are used as a colourant, flavour, and/or as a source of pungency (Corina *et al.*, 2015). The most common pepper names are chili, bell, red, green or just pepper (Faustino *et al.*, 2007). Peppers can be used fresh, dried, fermented, or as an oleoresin extract. They have both nutritional and nutraceutical importance. They contain anticoagulants that help to prevent blood clots that can lead to heart attacks (Muhammad *et al.*, 2011). In addition, several studies have demonstrated the antimicrobial activity of peppers (Cichewicz and Thorpe, 1996; Wahba *et al.*, 2010).

Capsicum, commonly known as pepper is a genus of plants from the Solanaceae family that have a variety of names depending on location and type. Genus *Capsicum* has five species that are commonly recognized as domesticated: *C. annuum, C. baccatum, C. chinense, C. frutescens,* and *C. pubescens. C. annuum* is mostly used commercially (Muhammad *et al.*, 2011). Fruits from the pungent hot type pepper plant are historically employed in traditional medicine and are currently being used in modern herbology and conventional medicines. Capsaicin, the predominant compound in pungent types of *Capsicum*, induces depletion of substance P and other neuropeptides from sensory nerve terminals (Altýnterim, 2013). A capsaicin cream has been introduced into dermatologic therapy and proven useful in preventing chronic pain associated with post-herpetic neuralgia, diabetic neuropathy, and other pain syndromes (Palevitch and Craker, 2012).

Most edible herbs regarded as spices are traditionally prepared through different methods. Sometimes the fresh material is directly consumed along with the meal or the herbs might be air-dried or alternatively exposed to the direct sunlight prior

to use while oven and microwave drying are considered newer methods. People are used to drying spicy plant material in order to keep the herbs for future cooking as well as reducing the risk of bacterial or fungal contamination (Vilela and Artur, 2008).

Drying is one of the most used traditional processes for food preservation, which promotes the concentration of the macronutrient content, eliminating the use of additives. It allows alteration of the original organoleptic properties, giving rise to new products and allowing their addition in different formulations, improving the sensorial aspect and quality of other foods (Vilela and Artur, 2009). Drying is a complex process involving simultaneous coupled transient heat, mass and momentum transport (Haghi and Amanifard, 2008). Dried foods are more concentrated than fresh foods with low moisture contents and can be stored at ambient temperatures for longer periods. Due to a considerable decrease in the water content of the material, dried foods have reduced microbiological activity with minimized physical and chemical changes (Araujo *et al*., 2004; Vega-Gálvez *et al*., 2007). Peppers, similar to other vegetables, are perishable resulting in high losses due to storage problems and marketing. An alternative to the consumption of fresh vegetables is their dried form, which allows their use during the off-season. However, food products are sensitive to drying temperatures and methods that can induce degradation (e.g., oxidation, loss of color, shrinkage or loss of texture) and change in the nutritional and functional properties of the products (Attanasio *et al*., 2004).

Since many herbs are used as dried form, drying process may affect their phenolic content and antioxidant activity. Therefore, it is necessary to investigate the effect of drying on the antioxidant activities of plants. Hence, this study investigates the effect of sun-drying on the antioxidant potentials of three varieties of *capscium species*.

MATERIALS AND METHODS

Collection and Preparation of Samples

The peppers used in the study were *C. annuum* var, *C. chinense* and *C. annuum*. Fresh samples of each pepper were purchased from a local market in Ede, Osun state, Nigeria and washed with tap water and then rinsed in distilled water to remove any debris. The samples were divided into two parts: one for fresh sample and the other for the dried sample. The fresh sample was ground using an electrical blender and kept in air tight container for further processing. The portion for the dried sample was sun-dried for 30 days, ground and kept in air tight container for processing.

Preparation of the Methanol Extract of *C. annuum* var, *C. chinense* and *C. annuum*

Extracts of both fresh and dried peppers were prepared using methanol as extracting solvent (Sun *et al*., 2007). The ground pepper (30 g) was extracted with 300 ml of 50% methanol (v/v) using Soxhlet apparatus for approximately 24 hr. Crude methanol extract of the samples was obtained by evaporating the extract to dryness using rotary apparatus.

Determination of Total Phenolic Content (TPC) of *C. annuum* var, *C. chinense* and *C. annuum*

The phenolic compounds content in the crude extract was determined according to the colorimetric method of Folin-Denis as described by Hatami *et al*. (2014). Briefly, 1 ml of each extract (1 mg/ml) was pipetted into a test tube and mixed with 1 ml of 95% ethanol and 5 ml of distilled water. To each sample, 0.5 ml of 50% (v/v) Folin–Ciocalteu reagent was added and mixed. After 5 min, 1 ml of 5% Na_2CO_3 was added to the reaction mixture and allowed to stand for 60 min. The absorbance was read at 725 nm. The values of total phenolics were expressed as garlic acid equivalents (mg garlic acid equivalent (GAE) per 100 g of sample). Standard curve was prepared using various concentrations of garlic acid in 95% ethanol. The analysis was conducted in triplicate.

Determination of Total Flavonoid Content (TFC) of *C. annuum* var, *C. chinense* and *C. annuum*

The method of formation of aluminium chloride complex assay was used as described by Chang *et al*., (2002) to determine the total flavonoid content of the extracts using quercetin as standard with slight modifications.

The reaction mixture contained 1 ml of methanol

solution of the extract (1 mg/ml) and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The mixture was incubated for an hour at room temperature. The absorbance was read at 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of quercetin and the calibration line was prepared. The concentration of flavonoids was interpolated from the calibration curve. The content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of QE/g of extract).

Evaluation of DPPH Radical-Scavenging Activity (DPPH) Activity of *C. annuum* var, *C. chinense* and *C. annuum*

DPPH method is based on the reduction in absorbance of the free radical DPPH (1, 1-diphenyl -2-picrylhydrazyl) by antioxidants at the visible wavelength of 517 nm. The DPPH radical scavenging activity of the extracts was carried out by the method described by Meda *et al.* (2005).

To 3 ml of 60 µM DPPH in ethanol, 250 µl of each extract (1 mg/ml) was added; the decrease in absorbance was monitored at 517 nm until a constant reading was obtained. The readings were compared with the control, which contained 250 µl of 95% ethanol instead of the extract. Garlic acid was used as standard.

The percentage inhibition of DPPH by extracts was calculated by using the following formula:

$$\% \text{ Inhibition} = \left(\frac{A_{517}^{\text{Control}} - A_{517}^{\text{Extract}}}{A_{517}^{\text{Control}}} \right) \times 100$$

Evaluation of Ferric Reducing Antioxidant Activity of *C. annuum* var, *C. chinense* and *C. annuum*

The ferric reducing power of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Zhao *et al.* (2008). Briefly, 100 µl of the extract (100–500 µg/ml) was mixed with 2.5 ml of 200 mmol/L phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50 °C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid (TCA) was added, and the tubes were centrifuged at 3,000 rpm for 15 min. Then, 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride. The absorbance of the reaction mixtures was measured at 700 nm. á-tocopherol was used as positive control and the ferric reducing antioxidant power was expressed as ìmol Fe(II)/g of the sample. The analysis was carried out in triplicate.

Statistical Analysis

Data were expressed as mean ± SD. Statistical differences at $p<0.05$ between the data were analyzed using one way ANOVA followed by Duncan's Multiple Range Test (DMRT) using SPSS 15.0 software.

RESULTS

Concentrations of Total Flavonoids (TF) and Total Phenolics (TP) of *C. annuum* var, *C. chinense* and *C. annuum*

Table 1 showed the results of the concentrations of total flavonoids and total phenolics in *C. annuum* var, *C. annuum* and *C. chinense*. The concentrations of flavonoids and phenolics were expressed as quercetin equivalent (QE/g) and garlic acid equivalent (GAE/g) respectively. There was a significant reduction in total flavonoids and total phenolics of the dried samples when compared to the fresh samples.

Table 1: Concentrations of Total Flavonoids (TF) and Total Phenolics (TP) of *C. Annuum* var, *C. chinense* and *C. annuum*

Sample Type	Pepper Variety	Total Phenolics mg/g GAE	Total Flavonoids mg/g QE
Fresh	*C. annuum* var	5.91 ± 0.22^{a}	3.80 ± 0.02^{f}
	C. chinense	6.9 ± 0.23^{b}	$3.91 + 0.08^{f}$
	C. annuum	6.67 ± 0.99^{b}	3.84 ± 0.08^{f}
Dried	*C. annuum* var	3.31 ± 0.72^{c}	1.26 ± 0.90^{d}
	C. chinense	3.59 ± 0.89^{c}	1.95 ± 0.07^{e}
	C. annuum	3.01 ± 0.17^{c}	1.23 ± 0.04^{d}

Values were represented as mean ± SD, n = 3.

Values of $p > 0.05$ in the same column were considered significant.

DPPH- Radical Scavenging Activity of *C. annuum var, C. chinense* and *C. annuum*

In table 2 is the result of the DPPH- radical scavenging activity of *C. annuum* var, *C. chinense* and *C. annuum*. There was significant reduction in the inhibitory properties of the fresh samples when compared to garlic acid. There was a significant reduction in inhibitory properties of the dried samples when compared to the fresh samples and garlic acid.

Table 2: DPPH- Radical Scavenging Activity of *C. annuum* var, *C. chinense* and *C. annuum*

Sample Type	Pepper Variety	% Inhibition
Fresh	*C. annuum* var	59.4 ± 0.5^{b}
	C. chinense	61.2 ± 0.6^{b}
	C. annuum	58.9 ± 0.2^{b}
Dried	*C. annuum* var	39.2 ± 0.5^{c}
	C. chinense	$42.\ 4 \pm 0.4^{c}$
	C. annuum	38.6 ± 0.6^{c}
Standard	Garlic Acid	70.8 ± 2.4^{a}

Values were represented as mean ± SD, n = 3.

Values of $p > 0.05$ in the same column were considered significant.

Ferric Reducing Antioxidant Potential (FRAP) of *C. annuum* var, *C. chinense* and *C. annuum*

The results of the Ferric Reducing Antioxidant Potential (FRAP) of *C. annuum* var, *C. chinense* and *C. annuum* are shown in table 3 There were significant reduction in the ferric reducing antioxidant potential of the fresh samples when compared to α-tocopherol while the dried samples showed no significant FRAP effect when compared to fresh samples and α-tocopherol.

Table 3: Ferric Reducing Antioxidant Potential of *C. annuum* var, *C. chinense* and *C. annuum*

Sample Type	Pepper Variety	μmol Fe(II)/g
Fresh	*C. annuum* var	588.56 ± 29.4^{c}
	C. chinense	691.34 ± 20.46^{d}
	C. annuum	$598.9 \pm 23.\ 82^{c}$
Dried	*C. annuum* var	370.22 ± 14.75^{b}
	C. chinense	392.34 ± 45.74^{b}
	C. annuum	358.6 ± 30.08^{b}
Standard	α-Tocopherol	956.44 ± 23.48^{a}

Values were represented as mean ± SD, n = 3.

Values of $p > 0.05$ in the same column were considered significant.

DISCUSSION

There are diverse factors that can greatly affect the phytochemical content such as polyphenolic compounds in plants. These include extraction solvent, pH, light, and heat (Akowuah *et al*., 2009). Also, the content of these active substances in plants may vary due to the location and origin of the plant, its growth phase and seasonal change (Young *et al*., 2005). The total flavonoids and total phenolics in plants are unstable compounds and their degradative reactions usually occur throughout the stages of formulation process of a dietary supplement (Akowuah *et al*., 2009).

Researchers have reported both negative and positive impacts of drying on the antioxidant capacity of medicinal plants. An increase in the phenolic content was observed in some studies while others observed a substantial loss of phenolic content in vegetables as a result of heat (Lima *et al*., 2009; Chipurura *et al*., 2010). The results of this study revealed a significant decrease in total phenolic and total flavonoid content in the dried sample of *C. annuum* var*, C. chinense* and *C. annuum*. This corroborates with the findings of Roy *et al*. (2007) who observed that reduced temperature of processing was found to preserve 80-100% of phenolic content in some vegetables. Lopez *et al*. (2010) also reported that an increase in drying temperature reduced the concentration of total phenolic of blueberry varieties when compared with the fresh sample. Long drying times associated with low process temperatures (e.g., 50, 60, and 70 °C) contribute to diminishing the protective effect of plants' extracts against oxidative damage to cells.

In constrast to the findings of this study, Chen *et al*. (2011) observed that when the citrus fruit (*Citrus sinensis* (L.) Osbeck) peels were dried at 50 and 60 °C, the total phenolic contents were significantly lower than those of fresh peels. However, the phenolic content gradually increased as drying temperature increased. The highest total phenolic content was in the peel dried at 100 °C. Its content was increased around two-fold compared with that of the fresh peel. In general, drying process resulted in a depletion of naturally occurring antioxidants in raw plant materials (Tomaino *et al*., 2005). Intense and/or prolonged thermal treatment may have significant effect on loss of natural antioxidants (Nicoli *et al*., 2012). A significant increase in polyphenols concentration observed at high temperature (e.g., 90 °C) in some studies was probably due to generation of different antioxidant compounds with a varying degree of antioxidant activity.

Flavonoids constitute the largest group of plant phenolics, accounting for over half of the eight thousand naturally occurring phenolic compounds (Balasundram *et al*., 2006). Currently, there is an increasing interest in flavonoids research due to the possibility of improved public health through diet, where preventive health care can be promoted through the consumption of fruit and vegetables (Ignat *et al*., 2011). Fruits and vegetables rich in flavonoids can be consumed either as fresh or processed products. However, major flavonoids present in fruits and vegetables, including flavonols, flavones, flavanones, flavanols, and anthocyanins, may be affected by different processing methods including drying (Kamiloglu *et al*., 2016). The results of the present study demonstrated that the fresh samples of the pepper variety possessed more flavonoid content than the dried samples.

The DPPH radical is long-lived organic nitrogen radical and has a deep purple colour. The assay principle is based on the reduction of the purple chromogen radical by antioxidant/reducing compounds to the corresponding pale yellow hydrazine (Brand-Williams *et al*., 1995). The ability of antioxidants can be estimated by measuring the decreasing of absorbance (Rahim *et al*., 2010). The result of the present study indicated that fresh samples of *C. annuum* var*, C. chinense* and *C. annuum* exhibited higher DPPH percentage inhibition. This may be due to the high amount of phenolics and flavonoids in the fresh pepper varieties. Drying temperature can affect the total antioxidant activity because most of antioxidant compounds are phenolic compounds and can be influenced by temperature (Thamer *et al*., 2018).

Lopez *et al*. (2010) investigated the DPPH-radical scavenging activity of blueberry varieties based on air-drying temperature. Dehydration at high temperatures (e.g., 80 and 90 °C) showed higher antioxidant activity rather than at low temperatures (e.g., 50, 60, and 70 °C). The result

of this study is not in agreement with the report. This behavior could be related to drying process at low temperatures which implies that long drying times may cause a decrease of antioxidant activity (Garau *et al*., 2007).

The FRAP assay is based on the ability of phenolics to reduce Fe^{3+} to Fe^{2+} (Thamer *et al*., 2018). The reducing capacity of compound may serve as a significant indicator of its potential antioxidant activity (Meir *et al*., 1995). A decrease in the ferric reducing antioxidant potential of the dried pepper samples was observed when compared with the fresh samples. This could be attributed to the decrease in the concentration of flavonoids and phenolics in the extracts as a result of sun-drying over a long period of time (Ansari *et al*., 2013).

CONCLUSION

The study concluded that dried samples of *C. annuum* var*, C. chinense* and *C. annuum* had a significant reduction in the concentration of total phenolics, flavonoids and DPPH-radical scavenging potentials and ferric reducing antioxidant potential when compared with the fresh samples. This could be attributed to oxidation of these antioxidant compounds as a result of sun drying. It is therefore recommended that consumers should feed on the fresh pepper varieties as they possess more antioxidant potential.. However, further studies should be carried out on the best method for the preservation of *Capsicum* species.

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