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Hot pepper (*Capsicum annuum*, Tepin and *Capsicum chinese*, Habanero) prevents Fe^{2+}-induced lipid peroxidation in brain – *in vitro*

G. Oboh [a,b,*], R.L. Puntel [b], J.B.T. Rocha [b]

[a] *Biochemistry Department, Federal University of Technology, P.M.B. 704 Akure, Nigeria*
[b] *Departamento de Quimica, Universidade de Federal Santa Maria (UFSM), Campus Universitário – Camobi, 97105-900 Santa Maria RS, Brazil*

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Abstract

Iron is an essential metal for normal cellular physiology, but excess iron results in cell injury; it reacts with superoxide anions ($O_2^{\cdot}$) and hydrogen peroxide (H_2O_2) to produce the hydroxyl radical ($OH^{\cdot}$) and other reactive oxygen species (ROS) which can cause damage to body cells. Free radical damage can be prevented by food rich in antioxidants such as fruit and vegetables. In the present study, the ability of aqueous extracts of ripe (red) and unripe (green) hot peppers [*Capsicum annuum*, Tepin (CAT) and *Capsicum chinese*, Habanero (CCH)] (3.3–16.7 mg/ml) to prevent 25 μM Fe^{2+}-induced lipid peroxidation in Rat's brain (*In vitro*) were assessed using TBARS (Thiobarbituric acid reactive species). The total phenol and vitamin C content, as well as Fe^{2+}-chelating ability, and the ability of the pepper extracts to prevent Fe^{2+}/H_2O_2-induced decomposition of deoxyribose was also determined. The results of the study revealed that incubating the brain tissues in the presence of 25 μM Fe^{2+} caused a significant increase ($p < 0.05$) in MDA (Malondialdehyde) production in the rat's brain (260%) when compared with the basal (100%). However, the pepper extracts (unripe and ripe) caused a significant decrease ($p < 0.05$) in the MDA production in both the basal and the Fe^{2+}-induced lipid peroxidation in the Rat's brain. However, CAT [ripe and unripe] had a significantly ($p < 0.05$) higher inhibitory effect on both basal and Fe^{2+}-induced lipid peroxidation in the brain tissues than that of CCH (ripe and unripe). In addition, CAT (ripe and unripe) had a significantly higher ($p < 0.05$) total phenol, vitamin C and Fe^{2+} chelating ability than CCH (ripe and unripe). The unripe CAT had a significantly ($p < 0.05$) higher total phenol, Fe^{2+} chelating ability and inhibitory effect on the basal and Fe^{2+}-induced lipid peroxidation in the brain tissues than the ripe pepper, while the reverse is the case with CCH where the red pepper had a higher values for the same parameters. However, ripe CAT and CCH had a significantly higher ($p < 0.05$) vitamin C content than the unripe; meanwhile both ripe and unripe peppers (CAT&CCH) did not significantly inhibit ($p < 0.05$) Fe^{2+}/H_2O_2-induced decomposition of deoxyribose (Fenton reaction). The inhibitory effect of the pepper on lipid peroxidation (basal and Fe^{2+} induced) and Fe^{2+} chelating effect of the extracts were dose dependent. It was therefore concluded that hot peppers prevent Fe^{2+}-induced lipid peroxidation, however CAT (ripe and unripe) are more potent inhibitors of Fe^{2+}-induced lipid peroxidation than CCH (unripe and ripe), meanwhile unripe CAT had the highest protective ability and this is probably due to its higher total phenol content and Fe^{2+} chelating ability.

Keywords: Pepper; Fe^{2+}; Rat; Brain

1. Introduction

During the past decade, it has been reported that the consumption of certain foods may have a positive effect on an individual's health. Foodstuffs supply not only energy, essential amino acids, fiber, vitamins, and minerals but also some active compounds such as antioxidants (tocopherols, carotenoids, vitamin C, phenolic compounds,

* Corresponding author. Address: Biochemistry Department, Federal University of Technology, P.M.B. 704 Akure, Nigeria. Tel.: +234 8035600192.
E-mail address: goboh2001@yahoo.com (G. Oboh).

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etc.) that may have different beneficial functions in the body. Dietary components, which are capable of acting as antioxidants, are likely to be beneficial by augmenting cellular defenses and protecting the cell against damage caused by free radicals, by acting as radical scavengers, reducing agents, potential complexes of prooxidant metals, and quenchers of singlet oxygen formation (Doblado et al., 2005; Gutteridge, 1993; Hochstein & Atallah, 1988; Oboh, 2005, 2006).

Iron is an essential metal for normal cellular physiology, but excess iron can result in cell injury. This is because it plays a catalytic role in the initiation of free radical reactions. The resulting oxyradicals have the potential to damage cellular lipids, nucleic acids, proteins and carbohydrates; the result is wide-ranging impairment in cellular function and integrity (Britton, Leicster, & Bacon, 2002). The mechanism by which iron can cause this deleterious effect is that Fe(II) can react with hydrogen peroxide (H_2O_2) to produce the hydroxyl radical ($OH^{\cdot}$) via the Fenton reaction, whereas superoxide can react with iron(III) to regenerate iron(II) that can participate in the Fenton reaction (Fraga & Oteiza, 2002; Harris et al., 1992). The overproduction of ROS can directly attack the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation. The inhibition of ion pump ATPases in plasma membranes can also occur as a result of iron-promoted formation of ROS and subsequent lipid peroxidation. Moreover, the reactive oxygen species can activate the transcription factors as nuclear factor-kB (NF-kB), which up-regulates the transcription of adhesion molecules, cytokines and enzymes, all involved in the inflammatory responses (Elmegeed, Ahmed, & Hussein, 2005).

For years researchers have known that free radicals can cause cell degeneration, especially in the brain. The brain and nervous system are particularly vulnerable to oxidative stress due to limited antioxidant capacity (Shulman, Rothman, Behar, & Hyder, 2004). The brain makes up about 2% of a person's mass but consumes 20% of their metabolic oxygen. The vast majority of this energy is used by the neurons (Shulman et al., 2004). Some brain cells, like neurons, cannot make glutathione, but instead rely on surrounding astrocyte cells to provide useable glutathione precursors, because the brain has limited access to the bulk of antioxidants produced by the body, neurons are the first cells to be affected by a shortage of antioxidants, and are most susceptible to oxidative stress (Perry, Norman, Litzburg, & Gelbard, 2004). High levels of both Cu and Fe, with relatively low levels of Zn and Mn, play a crucial role in brain cancer and in degenerative diseases of the brain (Parkinson's and Alzheimer's diseases, multiple sclerosis, etc.) (Johnson, 2001).

The human body is equipped with an antioxidant defense system that deactivates these highly reactive free radicals. Antioxidant enzymes (made in the body) and antioxidant nutrients (found in foods) soak up all the excess energy that these free radicals have, turning them into harmless particles that can be metabolized, so these antioxidant nutrients are functional components of food that have extra health benefits in the body (Oboh, 2005). Phenols, including flavonoids, can potentially protect body cells against the damage caused by reactive oxygen species (ROS). Much of the total antioxidant activity of fruits and vegetables is related to their phenolic content, not only to their vitamin C content. Research suggests that many flavonoids are more potent antioxidants than vitamins C and E (Mimica-Dukic, 2005; Oboh, 2005; Oboh & Akindahunsi, 2004). Natural polyphenols exert their beneficial health effects by their antioxidant activity. These compounds are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases (Amic, Davidovic-Amic, Beslo, & Trinajstic, 2003; Alia, Horcajo, Bravo, & Goya, 2003; Oboh, 2006; van Acker, van Balen, van den Berg, Bast, & van der Vijgh, 1998; Sestili, Guidarelli, Dacha, & Cantoni, 1998).

Pepper fruits (*Capsicum annuum* L.) are important vegetables used as foods and as spice. Peppers are a good source of vitamins C and E (Daood, Vinkler, Markus, Hebshi, & Biacs, 1996; Palevitch & Craker, 1995) as well as provitamin A and carotenoids compounds with well-known antioxidant properties (Krinsky, 1994; Krinsky, 2001; Matsufuji, Nakamura, Chino, & Takeda, 1998). Green peppers are often harvested before they are ripe, and changes in the maturity may affect the content of phytonutrients (Marin, Ferreres, Tomas-Barberan, & Gil, 2004), which play important roles in the diet any antioxidant intake. Fresh peppers have higher content of vitamin C (Vanderslice, Higgs, Hayes, & Block, 1990), and the carotenoid pigments in fresh peppers have been widely studied to improve colour retention during processing and storage (Markus, Daood, Kapitany, & Biacs, 1999; Mınguez-Mosquera & Hornero-Mendez, 1993; Mınguez-Mosquera, Jaren-Galan, & Garrido-Fernandez, 1994; Mınguez-Mosquera & Hornero-Mendez, 1994).

Capsicum chinese, Habanero are said to be the hottest of all the peppers – up to 40 times hotter than a Jalapeño! There are many different forms of habaneros: typical habaneros are orange when ripe, but some ripen to red, some to brown, some to white, and some to purple. While *Capsicum annum* var. Tepin are little gems and are also called bird's eye or bird peppers. They are considered to be hotter than habaneros, and are close to the ancestor of all the cultivated *C. annuum* varieties. They are similar to chile piquins, but are pea shaped instead of bullet shaped. They grow wild in Mexico and the southern US (including Florida). Although a lot has been reported on the chemical characteristics of antioxidant phytoconstituents in peppers, there is no information on the biological activity of pepper (*in vitro and in vivo*); this study therefore sought to compare the protective properties of aqueous extracts of two varieties of hot pepper [*Capsicum annum*, Tepin and *Capsicum chinese*, Habanero] on Fe^{2+}-induced lipid peroxidation in Rat's brain homogenates – *In vitro* in their ripe and unripe state.

2. Materials and methods

2.1. Materials

Fresh samples of two varieties of hot peppers, namely *Capsicum annuum* var. Tepin and *Capsicum chinese* var. Habanero, were collected from vegetable gardens in Camobi, Santa Maria RS, Brazil. The authentication of the pepper was carried out in Departamento de Biologia, Universidade Federal de Santa Maria, Santa Maria RS, Brazil. All the chemicals used were analytical grade, while the water was glass distilled. The handling and the use of the animals were in accordance with NIH Guide for the care and use of laboratory animals. In the experiments Wister strain albino rats weighing 200–230 g were collected from the breeding colony of Departamento de Biologia, Universidade Federal de Santa Maria, Santa Maria RS, Brazil. They were maintained at 25 °C, on a 12 h light/12 h dark cycle, with free access to food and water.

2.2. Aqueous extract preparation

The inedible portions of the pepper were removed from the edible portion, this edible portion was subsequently washed in distilled water. The aqueous extract of the fresh pepper was subsequently prepared by homogenizing 1 g of the pepper in 20 ml-distilled water, and the homogenates were centrifuged at 2000 rpm for 10 min. The supernatant was used for the Fe^{2+} chelation and lipid peroxidation assay.

2.3. Preparation of brain homogenates

The rats were decapitated under mild diethyl ether anaesthesia and the cerebral tissue (whole brain) was rapidly dissected and placed on ice and weighed. These tissues were subsequently homogenized in cold saline (1/10 w/v) with about 10 up and down strokes at approximately 1200 rev/min in a Teflon-glass homogenizer. The homogenate was centrifuged for 10 min at 3000*g* to yield a pellet that was discarded and a low-speed supernatant (S1) containing mainly water, proteins and lipids (cholesterol, galactolipid, individual phospholipids, gangliosides), DNA and RNA, which was kept and collected for lipid peroxidation assay (Belle, Dalmolin, Fonini, Rubim, & Rocha, 2004).

2.4. Lipid peroxidation and thiobarbibutric acid reactions

The lipid peroxidation assay carried out using the modified method of Ohkawa, Ohishi, and Yagi (1979). Briefly 100 μl S1 fraction was mixed with a reaction mixture containing 30 μl of 0.1 M pH 7.4 Tris–HCl buffer, pepper extract (0–100 μl) and 30 μl of 250 μM freshly prepared $FeSO_4$ and the volume was made up to 300 μl by water before incubation at 37 °C for 1 h. The colour reaction was developed by adding 300 μl 8.1% SDS (sodium dodecyl sulphate) to the reaction mixture containing S1, this was subsequently followed by the addition of 600 μl of acetic acid/HCl (pH 3.4) mixture and 600 μl 0.8% TBA (Thiobarbituric acid). This mixture was incubated at 100 °C for 1 h. TBARS (Thiobarbituric acid reactive species) produced were measured at 532 nm and the absorbance was compared with that of the standard curve using MDA (Malondialdehyde).

2.5. Total phenol determination

The total phenol content was determined by mixing 0.5 ml of the aqueous extract of the pepper with 2.5 ml 10% Folin–Cioalteu's reagent (v/v) and 2.0 ml of 7.5% sodium carbonate was subsequently added. The reaction mixture was incubated at 45 °C for 40 min, and the absorbance was measured at 765 nm in the spectrophotometer, Gallic acid was used as standard phenol (Singleton, Orthofer, & Lamuela-Raventos, 1999).

2.6. Vitamin C determination

The vitamin C was determined using the method of Benderitter et al. (1998). Briefly 75 μl of DNPH (2 g of dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSo_4 \cdot 5H_2O$ in 100 ml 5M H_2SO_4) was added to 500 μl reaction mixture (300 μl of the pepper extracts with 100 μl 13.3% TCA and water, respectively). The reaction mixture was subsequently incubated for 3 h at 37 °C, then 0.5 ml H_2SO_4 65% (v/v) was added to the medium, and the absorbance was measured at 520 nm, and the Vitamin C content of the sample was subsequently calculated, using a vitamin C standard curve.

2.7. Fe^{2+} chelation assay

The ability of the aqueous extract to chelate Fe^{2+} was determined using a modified method of Minotti and Aust (1987) with a slight modification by Puntel, Nogueira, and Rocha (2005). Briefly 150 μl of freshly prepared 500 μM $FeSO_4$ was added to a reaction mixture containing 168 μl of 0.1 M Tris–HCl (pH 7.4), 218 μl saline and the aqueous extract of the pepper (0–25 μl). The reaction mixture was incubated for 5 min, before the addition of 13 μl of 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in the spectrophotometer.

2.8. Degradation of deoxyribose (Fenton's reaction)

The ability of the aqueous extract of the pepper to prevent Fe^{2+}/ H_2O_2-induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge (1981). Briefly, freshly prepared aqueous extract (0–100 μl) was added to a reaction mixture containing 120 μl 20 mM deoxyribose, 400 μl 0.1 M phosphate buffer, 40 μl 20 mM hydrogen peroxide and 40 μl 500 μM $FeSO_4$, and the volume was made up to 800 μl with distilled water.

The reaction mixture was incubated at 37 °C for 30 min, and the reaction was then stopped by the addition of 0.5 ml of 2.8% TCA (Trichloroacetic acid), this was followed by the addition of 0.4 ml of 0.6% TBA solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in spectrophotometer.

2.9. Analysis of data

The result of the replicates were pooled and expressed as mean ± standard error (S.E.) (Zar, 1984). A one way analysis of variance (ANOVA) and the Least Significance Difference (LSD) were carried out. Significance was accepted at $p \leqslant 0.05$. The EC_{50} (extract concentration that will cause 50% inhibition of lipid peroxidation in the Rat's brain) of the pepper extracts were determined using the method of Dixon and Massey (1969).

3. Results and discussion

Neurodegenerative diseases and aging processes associated with Fe accumulation could be managed prevented by Fe chelators (Fraga & Oteiza, 2002; Weinberg, 2005). Indeed, one of the ways polyphenols exhibit their antioxidant activity is by chelating Fe. Accordingly, the results of the total phenol content of ripe and unripe hot peppers (CAT and CCH) are presented in Table 1. The result of the study revealed that *Capsicum annuum*, Tepin [ripe (208.5) and unripe (236.5)] had a significantly higher ($p < 0.05$) total phenol content than *Capsicum chinese*, Habanero [ripe (103.2) and unripe (73.7)]. However, the total phenol content of *Capsicum annuum*, Tepin was higher than the total phenol content reported by Chu, Sun, Wu, and Liu (2002) for peppers; while that of *Capsicum chinese*, Habanero was within the same range with the value reported by Chu et al. (2002) for red peppers. The phenol content of *Capsicum chinese*, Habanero is lower than phenol content of some tropical leafy vegetables (Oboh, 2005; Oboh & Akindahunsi, 2004) and commonly consumed fruits (except lemon, banana, peach, orange, pear and grape fruit that had the same total phenol range as pepper) (Chu et al., 2002; Sun, Chu, Wu, & Liu, 2002). However, the phenol content of *Capsicum annuum*, Tepin was within the same range of that of some tropical green leafy vegetables (Oboh, 2005; Oboh & Akindahunsi, 2004), strawberry and red grape; but higher than that of banana, pear, peach, orange and grapefruit; and lower than that of cranberry and apple (Sun et al., 2002).

Table 1
Total phenol content and vitamin C content of some hot pepper

Sample	Vitamin C (μg/g)	Total phenol (mg/100 g gallic acid equivalent)
Capsicum annuum, Tepin		
Red	332.5 ± 6.7^{a}	210.0 ± 5.2^{b}
Green	250.2 ± 10.0^{b}	226.3 ± 6.5^{a}
Capsicum chinese, Habanero		
Red	152.5 ± 5.6^{c}	103.2 ± 8.5^{c}
Green	103.6 ± 7.5^{d}	73.7 ± 5.5^{d}

Values represent means of triplicate.
Values with the same alphabet along the same column are not significantly different ($p > 0.05$).

The trend in the total phenol content *Capsicum annuum*, Tepin with maturity is in agreement with the trend reported by Marin et al. (2004) for *Capsicum annuum* L. cv. Vergasa (a variety of sweet pepper), where the green pepper had higher total phenol content than the red pepper, while the reverse is the case with *Capsicum chinese*, Habanero. However, the trend in the phenolic content of *Capsicum chinese*, Habanero with maturity agrees with the report of Materska and Perucka (2005) on some varieties of hot pepper [*Capsicum annum* L. (Bronowicka Ostra, Cyklon, Tomado and Tajfun)] where the red pepper had higher phenol content and antioxidant activity; while the reverse is the case with *Capsicum annuum*, Tepin. The results of the vitamin C content of the pepper are also shown in Table 1, the result revealed that *Capsicum annuum*, Tepin [ripe (330 μg/g), unripe (256 μg/g)] had a significantly higher ($p < 0.05$) vitamin C content than that of *Capsicum chinese*, Habanero [ripe (153 μg/g), unripe (108 μg/g)]. These values were generally lower than the vitamin C content of *Capsicum annuum* L. cv. Vergasa (Marin et al., 2004) and what Chu et al. (2002) reported for red pepper. However, ripe CAT and CCH had a significantly higher ($p < 0.05$) vitamin C content than the unripe pepper. This trend is in agreement with what Marin et al. (2004) reported for *Capsicum annuum* L. cv. Vergasa, in that the red pepper had higher vitamin C content than green pepper.

However, aqueous extracts of both peppers (ripe and unripe) significantly ($p < 0.05$) inhibit lipid peroxidation in Rat's brain in a dose-dependent manner (Fig. 1a and b). However, CAT had a higher inhibitory effect on lipid peroxidation in Rat's brain than CCH (Table 2), especially at higher extract concentration (8.3 mg/ml). Furthermore, the unripe pepper [CAT (1.2 mg/ml) and CCH (1.5)] extracts had a higher EC_{50} (extract concentration that caused 50% inhibition of lipid peroxidation in the Rat's brain tissues) than the ripe pepper [CAT (1.3 mg/ml) and CCH (2.8)] (Table 2).

The protective action of the aqueous extract of the peppers could be attributed to the presence of antioxidants, especially ascorbic acid and phenols (Chu et al., 2002; Matsufuji et al., 1998). Hot cultivars are rich in capsaicinoids alkaloids with pharmacological properties giving the specific taste to pepper fruit (Daood et al., 1996). There are also present flavonoids and phenolic compounds. The presence of derivatives of cinnamic acid and flavonoids has been found in pepper fruit; however, numerous studies have conclusively shown that the majority of the antioxidant activity may be from compounds such as flavonoids, isoflavone, flavones, anthocyanin, catechin and isocatechin rather than from vitamins C, E and β-carotene (Chu et al., 2002; Marin et al., 2004; Materska & Perucka, 2005; Sun

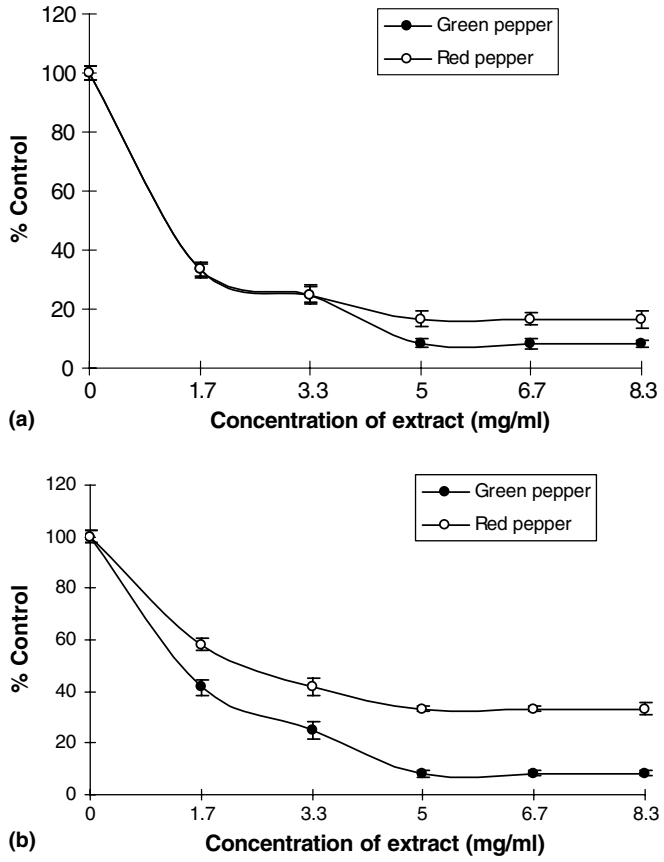

Fig. 1. (a) Inhibition of lipid peroxidation in Rat's brain by aqueous extract of red and green *Capsicum annuum*, Tepin. (b) Inhibition of lipid peroxidation in Rat's brain homogenates by aqueous extract of red and green *Capsicum chinese* Habanero.

Table 2
EC_{50} of some hot pepper

Sample	EC_{50} (mg/ml)
Capsicum annuum, Tepin	
Red	1.3 ± 0.1^{a}
Green	1.2 ± 0.3^{a}
Capsicum chinese, Habanero	
Red	2.8 ± 0.3^{b}
Green	1.5 ± 0.2^{a}

Values represent means of triplicate.
Values with the same alphabet along the same column are not significantly different ($p > 0.05$).

et al., 2002). However, the marked difference between the protective effects of both the ripe and unripe pepper of the same variety at higher concentration could be attributed to the fact that Peppers suffer a profound physiological change during the course of ripening with the conversion of existing pigments (Marin et al., 2004; Materska & Perucka, 2005).

The result of the inhibitory effect of aqueous extract of the two varieties of peppers (ripe and unripe) on Fe^{2+}-induced lipid peroxidation in Rat's brain are shown in Fig. 2a and b. The results clearly show that incubation of the Rat's brain in the presence of 25 μM Fe^{2+} caused a significant increase ($p < 0.05$) in the MDA contents of the Rat's brain when compared with the basal brain homogenates. The increased lipid peroxidation in the presence of Fe^{2+} could be attributed to the fact that Fe^{2+} can catalyze one-electron transfer reactions that generate reactive oxygen species, such us the reactive OH**.**, which is formed from H_2O_2 through the Fenton reaction. Iron also decomposes lipid peroxides, thus generating peroxyl and alkoxyl radicals, which favors the propagation of lipid oxidation (Zago, Verstraeten, & Oteiza, 2000). Iron overload is a less frequent condition, but high contents of tissue iron has been associated with several pathological conditions, including liver and heart disease (Milman et al., 2001; Rasmussen et al., 2001), cancer (Beckman et al., 1999; Parkkila, Niemela, Savolainen, & Koistinen, 2001), neurodegenerative disorders (Berg et al., 2001; Sayre, Perry, Atwood, & Smith, 2000), diabetes (Perez de Nanclares et al., 2000), hormonal abnormalities (Wilkinson, 1996), and immune system abnormalities (Walker and Walker, 2000).

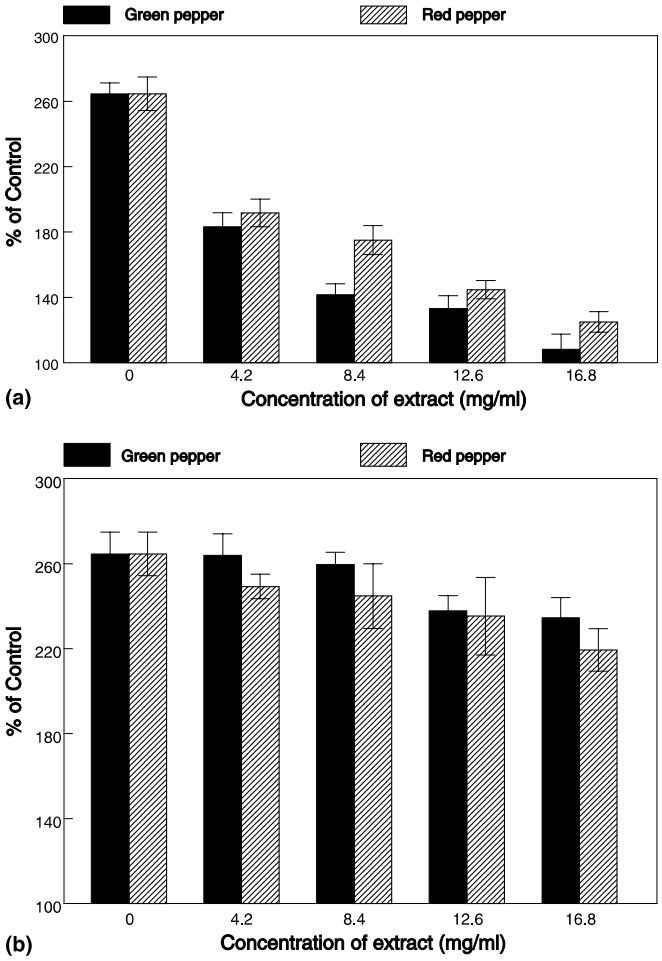

Fig. 2. (a) Inhibition of Fe^{2+}-induced lipid peroxidation in Rat's brain homogenates by aqueous extract of red and green *Capsicum annuum*, Tepin. (b) Inhibition of Fe^{2+}-induced lipid peroxidation in Rat's brain homogenates by aqueous extract of red and green *Capsicum chinese*, Habanero.

However, extracts from both peppers (ripe and unripe) caused a significant decrease ($p < 0.05$) in the brain MDA levels, during the Fe^{2+}-induced lipid peroxidation in Rat's brain tissues in a dose dependent manner. Aqueous extract from unripe CAT had higher inhibitory effect on the Fe^{2+}-induced lipid peroxidation in the Rat's brain homogenates than the red pepper extract (Fig. 2a). Conversely, the aqueous extract of the red *Capsicum chinese*, Habanero (CCH) inhibited Fe^{2+}-induced lipid peroxidation more than green pepper extract of the same pepper as shown in Fig. 2b. Comparison of Fig. 2a and b revealed that the extracts of red and green *Capsicum annuum*, Tepin (216.7–108.3%) inhibited Fe^{2+}-induced lipid peroxidation in Rat's brain tissues more than that of *Capsicum chinese*, Habanero (254.0–219.4%). The decrease in the Fe^{2+}-induced lipid peroxidation in the Rat's brain homogenates in the presence of the extracts could be as a result of the ability of the antioxidant phytochemicals in extracts to chelate Fe^{2+} and scavenge free radicals produced by the Fe^{2+} catalyzed production of reactive oxygen species (ROS) in the Rat's brain homogenates (Marin et al., 2004; Materska & Perucka, 2005; Oboh, 2005; Oboh & Akindahunsi, 2004; Oboh, 2006).

In order to provide an explanation for the inhibition of Fe^{2+}-induced lipid peroxidation in Rat's brain tissue, the Fe^{2+}-chelating ability of the water extractable phytochemicals in the peppers were determined and the results are shown in Fig. 3a and b. The aqueous extract of *Capsicum annuum*, Tepin [unripe (78.1–90.1%), ripe (64.5–84.6%)] had higher Fe^{2+} chelating ability than *Capsicum chinese*, Habanero [unripe (57.4–82.5%), ripe (62.5–83.5)] at the concentration tested (3.3–16.7 mg/ml). However, the aqueous extract of the unripe CAT had a significantly higher ($p < 0.05$) chelating ability than the aqueous extract of the red pepper at the lowest concentration tested (Fig. 3a). Conversely, the aqueous extract of red CCH had higher Fe^{2+} chelating ability than green CCH extract (Fig. 3b); however, there was no significant difference ($p > 0.05$) in the Fe^{2+} -chelating ability of the extract at higher concentration. Furthermore, *Capsicum annuum*, Tepin (ripe and unripe) extract had a higher Fe^{2+}-chelating effect than *Capsicum chinese*, Habanero (ripe and unripe) extract at all the concentration of the extract tested in this experiment.

It is worth noting, that there was an agreement between Fig. 2a and b and Fig. 3a and b, in that, extracts with higher Fe^{2+} chelating ability in Fig. 3a and b, had higher inhibitory effect on the Fe^{2+}-induced lipid peroxidation Rat's brain homogenates *In vitro* (Fig. 2a and b). This implies that the higher Fe^{2+}-chelating ability of *Capsicum annuum*, Tepin extract (ripe and unripe) in comparison with *Capsicum chinese*, Habanero (ripe and unripe) could have accounted for the higher inhibition of Fe^{2+}-induced lipid peroxidation in the rat's brain homogenates. Likewise, the trend in the stage of maturity on the inhibition of Fe^{2+}-induced lipid peroxidation and chelating activity are in agreement in both peppers; green CAT extract with the highest Fe^{2+} chelating effect had the highest inhibitory effect on Fe^{2+}-induced lipid peroxidation, while green CCH extract with the least Fe^{2+} chelating ability at all the concentrations tested had the least inhibitory effect on Fe^{2+}-induced lipid peroxidation. The difference in the inhibition of both varieties could be because of the difference in the antioxidant phytochemical composition of the pepper. However, the difference in the Fe^{2+} chelating effect and the ability of the extracts to prevent Fe^{2+}-induced lipid peroxidation in Rat's brain homogenates may be due to the fact that the ripening process in peppers could cause physiological changes in the antioxidant phytochemicals (Marin et al., 2004; Mınguez-Mosquera et al., 1994). However, while the ripening process leads to a decrease in the antioxidant properties of *Capsicum annuum*, Tepin, this process caused an increase in the antioxidant properties of *Capsicum chinese*, Habanero.

Furthermore, there was an agreement between the total phenol content (Table 1), Fe^{2+} chelating ability (Fig. 3a and b) and inhibition of Fe^{2+}-induced lipid peroxidation in the Rat's brain (Fig. 2a and b). Green *Capsicum annuum*, Tepin extract with the highest total phenol content had the highest Fe^{2+} chelating ability and inhibitory effect on the Fe^{2+}-induced lipid peroxidation in Rat's brain tissues,

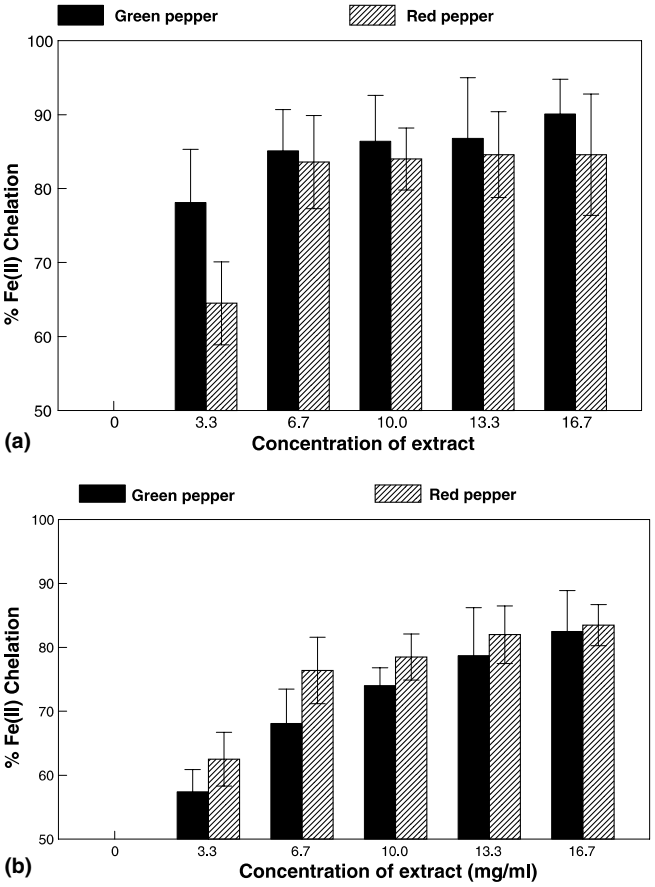

Fig. 3. (a) Fe^{2+} chelating ability of aqueous extract of red and green *Capsicum annuum*, Tepin. (b) Fe^{2+} chelating ability of aqueous extract of red and green *Capsicum chinese*, Habanero.

while green *Capsicum chinese*, Habanero with the lowest total phenol content, had the least Fe^{2+}-chelating and inhibitory effect on the Fe^{2+}-induced lipid peroxidation in the Rat's brain tissue. Conversely, the result of the vitamin C content of the peppers were not in agreement with the ability of the pepper extracts to inhibit lipid peroxidation in both the basal and Fe^{2+}-induced lipid peroxidation in Rat's brain. Therefore, the polyphenols present in the pepper could have been responsible for the inhibition of the Fe^{2+}-induced lipid peroxidation.

As shown in Fig. 4a and b, the pepper extracts did not significantly ($p > 0.05$) inhibit the decomposition of deoxyribose (Fenton's reaction). However, the reason for the inability of the extracts to significantly ($p < 0.05$) prevent deoxyribose decomposition (Fenton's reaction) could not be categorically stated, however it will not be unlikely that that the complex formed between the extract and Fe, could be chemically active in Fenton's reaction but not as an initiator of lipid peroxidation. This clearly indicates that, in addition to the already established free radical scavenging ability of pepper (Chu et al., 2002; Marin et al., 2004; Materska & Perucka, 2005), Fe^{2+} chelating ability could be a major contributory mechanism by which hot pepper prevents lipid peroxidation rather than by OH**.** radical scavenging; OH**.** is produced during Fe^{2+} catalyzed decomposition of hydrogen peroxide (Fraga & Oteiza, 2002).

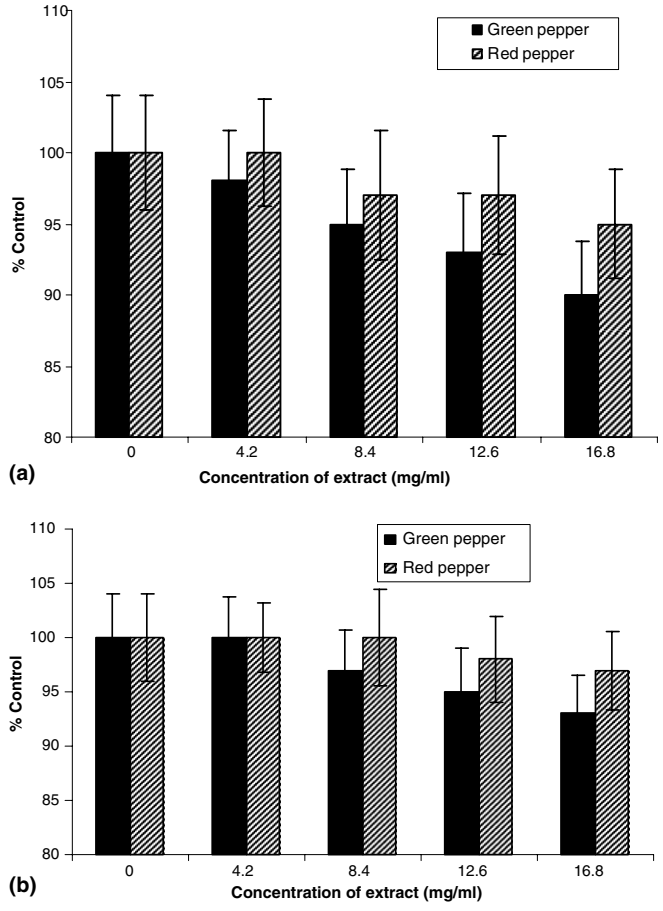

Fig. 4. (a) Inhibition of deoxyribose decomposition by *Capsicum annuum*, Tepin. (b) Inhibition of deoxyribose decomposition by *Capsicum chinese*, Habanero.

4. Conclusion

It was therefore concluded that hot pepper prevents Fe^{2+}-induced lipid peroxidation in Rat's brain – *In vitro*, however CAT (ripe and unripe) are more potent inhibitor of Fe^{2+}-induced lipid peroxidation than CCH (unripe and ripe), meanwhile unripe CAT had the highest protective ability and this is probably due to its higher total phenol content and Fe^{2+} chelating ability; furthermore while ripening will decrease the antioxidant properties of CAT, it increases that of CCH.

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