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ORIGINAL PAPER

Influence of Extraction Solvents on Antioxidant Activity and the Content of Bioactive Compounds in Non-pungent Peppers

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Abstract Bioactive compounds in foods have been shown to maintain human health. However, the relative amounts of bioactive compounds and the variation in the amounts are still poorly understood. In this study, the efficacy of different extraction solvents (hexane, ethyl acetate, acetone, methanol, and a methanol:water mixture), as well as the levels of certain bioactive compounds in non-pungent pepper cultivars (TMH, TMJ, PA137, and B58) were investigated using high-performance liquid chromatography (HPLC). Antioxidant activities were determined using 2,2,-diphenyl-1-picrylhydrazyl (DPPH), reducing power, and deoxyribose degradation. Hexane extracts had the highest level of carotenoids (47.2–628.8 μg/g), and methanol extracts contained maximum flavonoids (24.9–152.2 μg/g) in four different cultivars. Higher DPPH scavenging activity was found in the hexane extracts from TMH, TMJ, PA137, and B58 (IC_{50} value: 0.67, 0.74, 0.55, and 0.48 μg/ml, respectively), whereas the reducing power was high in ethyl acetate and acetone extracts. Inhibition of deoxyribose degradation was highest in methanolic extracts from TMH, TMJ, PA137, and B58 (51.2, 49.5, 52.6, and 47.4 %, respectively). These data demonstrate that solvent chemical properties such as polarity can differentially impact the efficiency with which different bioactive compounds are recovered from foods, and this could lead to differences in estimated biological activity such as antioxidant capacity.

Keywords Pepper · Bioactive compounds · Total phenolics · DPPH · Reducing power · Deoxyribose degradation

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Introduction

In recent years, awareness of the benefits of functional foods and interest in the discovery of natural bioactive compounds has risen substantially [1, 2]. Numerous plant secondary compounds, with demonstrated or proposed bioactivities, have been described in many foods. Antioxidant activity, the best well-known biological activity of these compounds, can help protect biological systems from oxidative stress caused by free radicals. However, in the human body, food antioxidants are converted by conjugation with hydroxyl groups to metabolites that are rapidly excreted [3, 4], due to their increased solubility [5]. Since estimating the exact amount of bioavailable antioxidants is complex, a comprehensive study of bioactive compounds in dietary materials will likely be required to substantially improve our understanding of food components [6]. Bioactive compounds such as ascorbic acid, carotenoids, and flavonoids occur naturally in many foods including fruits and vegetables such as peppers [7]. The diversity and levels of each bioactive compound in different foods are determined by both genetic and environmental factors [8]. In addition, phenolic compounds in varying amounts are one of the major components of antioxidant activity [9]. Vega-Gálvez et al. [10] reported that total phenolics in red fresh peppers were higher than in dried peppers. Sun et al. [11], found that sweet peppers with red, orange, yellow, and green colors contained different contents of total phenolics [11], while in another study,

green sweet peppers showed 6-fold variation in total phenolics among ten genotypes [12]. In addition to natural variation, the relative levels of these compounds can vary depending on the assay procedures used to estimate their concentrations in foods. Quantifying the levels of bioactive compounds is complicated by the fact that different food components can bind and inactivate compounds of interest, thereby yielding inaccurate measurements of levels [13, 14].

The choice of extraction solvents can influence the accuracy of measurements of the concentrations of bioactive compounds [15, 16]. Limited experimental data indicates that the concentration and activity of bioactive compounds in natural foods may be directly related to solvent properties such as lipophilic and hydrophilic solvents [11, 17] and their respective polarity. Carotenoids, being lipophilic, are extracted in non-polar solvents, but flavonoids, being hydrophilic, are extracted more in polar solvents. Previous studies have measured the antioxidant capacity of pepper extracts, comparing the levels of bioactive compounds. In these studies, the antioxidant capacity of pepper extracts, that is the ability to scavenge free radicals, was measured by a number of methods such as oxygen radical absorbance capacity [18], Trolox equivalent antioxidant activity [19], ferric reducing antioxidant property [19, 20] and the DPPH method [21]. However, these studies measured the antioxidant activities using single extraction solvent without comparing solvent polarity, which would affect the levels of bioactive compounds and antioxidant capacity. The objective of the present study was to determine the extraction efficiency of bioactive compounds from four pepper cultivars using five extraction solvents with different polarities. The antioxidant activities of the resultant extracts were evaluated using DPPH, reducing power, and degradation of deoxyribose. The results were correlated with total phenolics, carotenoids, and flavonoids.

Materials and Methods

Chemicals

Folin-Ciocalteu reagent was purchased from Biomedicals (Illkirch, France), and capsanthin (CAS 465-42-9) was obtained from ChromaDex (Irvine, CA, USA). 2,2,-Diphenyl-1-picrylhydrazyl (DPPH, CAS 56537-16-7), potassium ferricyanide (Purity 99 %; CAS 13746-66-2), trichloroacetic acid (Purity≥99 %; CAS 76-03-9), ferric chloride (Purity 97 %; CAS 7705-08-0), 2-deoxy-D-ribose (Purity 97 %; CAS 533-67-5), thiobarbituric acid (Purity 98 %; CAS 504-17-6), β-carotene (Purity≥93 %; CAS 7235-40-7), quercetin (Purity≥98 %; CAS 74893-81-5), luteolin (Purity~98 %; CAS 491-70-3), kaempferol (Purity≥ 90 %; CAS 66428-89-5), apigenin (Purity~95 %; CAS 520-36-5), and (+)-catechin (Purity≥99 %; CAS 7295-85-4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide 30 % (CAS 7722-84-1) was obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA). HPLC grade methanol and tert-butyl methyl ether were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Plant Materials

Non-pungent peppers, Habanero (*Capsicum chinense* cv. 'TMH'), Jalapeno (*C. annuum* cv. 'TMJ'), and Paprika (*C. annuum* cvs. 'PA137' and 'B58'), were grown in a greenhouse and used in this study. The pepper cultivars were developed as part of a genetic-improvement program at the Vegetable and Fruits Improvement Center of Texas A&M University at College Station, Texas. The cultivars were planted in spring and mature pepper samples were harvested in summer.

Sample Preparation

The mature peppers were ground, stored at −80 °C, and freeze dried. The freeze-dried samples (20 g) were extracted in Soxhlet extractors using 500 ml each of hexane, ethyl acetate, acetone, methanol (MeOH), and MeOH:water (80:20, v/v) in succession, according to our previous publication [22]. Extraction solvents used in this study were chosen to represent a full range of solvent polarity. The extraction solvents used were hexane, ethyl acetate, acetone, MeOH, and MeOH:water (80:20) in order of increasing polarity. If a sample contains polar bioactives and the polar solvents such as MeOH, and MeOH: water (80:20) will extract more efficiently the target compounds. Hexane more effectively extracts carotenoids than other solvents. On the other hand methanol and MeOH: Water (80:20) solvents are commonly used to extract flavonoids and total phenolics. Therefore, these five extraction solvents were chosen in this study. Extractions were conducted for 10 h at 60 °C to achieve maximum yield and 500 ml of each extraction solvent was used for 20 g of dried pepper. After extraction with each solvent, the extracts were concentrated using a rotary evaporator, and the next solvent extraction was conducted in the same way. All concentrated extracts were freeze-dried, and the yield of each extract was calculated.

High-Performance Liquid Chromatography (HPLC) Conditions

Capsanthin and β-carotene from pepper extracts were quantified by HPLC using authentic capsanthin and β-carotene as external standards. Stock solutions of capsanthin (5.6, 11.2, 22.5, 45.0, and 90.0 μg/ml) and β-carotene and (5.6, 11.2,

22.5, 45.0, and 90.0 μg/ml) were used for the calibration curve. The Elmer HPLC (Salem, MA, USA) system with a C_{30} YMC Carotenoid column (150×4.6 mm ID, 3 μm, particle size), a Nelson 900 autosampler, and photo-diode array detector was set at 450 nm. The mobile phase consisted of MeOH (A) and tert-butyl methyl ether (B) with a flow rate of 0.8 ml/min. Gradient elution was as follows: 0–80 % B (0–15 min), 80–100 % B (15–20 min), and 100–0 % B (20–25 min). Since capsanthin and β-carotene are the most commonly detected carotenoids in peppers, the contents of capsanthin and β-carotene were used to represent carotenoids in this study.

Authentic flavonoid standards (quercetin, luteolin, kaempferol, and apigenin) were used for quantification. Quercetin (9.3, 18.7, 37.5, 75.0, and 150.0 μg/ml), luteolin (3.1, 6.2, 12.5, 25.0 and 50.0 μg/ml), kaempferol (3.1, 6.2, 12.5, 25.0, and 50.0 μg/ml) and apigenin (4.8, 9.7, 19.5, 39, and 78 μg/ml) were used for a calibration curves. Flavonoids were determined by using the same HPLC system with a C_{18} Gemini column (250×4.6 mm ID, 5 μm particle size) at 360 nm [23]. The eluent with 0.03 M phosphoric acid in water (A) and MeOH (B) was carried out at a flow rate of 1 ml/min. The gradient was as follows: 40–100 % B (0–10 min), 100 % B (10–15 min), and 100–40 % B (15–20 min). For the quantification of flavonoid aglycones, the samples were hydrolyzed. A 6 ml aliquot of the extract was mixed with 3 ml of 3 M HCl, and the mixture was kept at 95 °C in a water bath for 1 h. The hydrolyzed solution was cooled to room temperature and filtered through a 0.45 μm membrane before HPLC analysis.

Total Phenolic Content

The concentration of total phenolics in extracts was determined using the Folin-Ciocalteu (FC) method [24]. The hydrolyzed sample (100 μl) was adjusted to 10 ml with water. Diluted FC reagent (500 μl) was added, and the sample was kept at room temperature. After 10 min, 1000 μl of sodium carbonate was added to the mixture, and the mixture was incubated at 23 °C for 20 min. The absorbance of blue color was measured at 760 nm using a 96-well plate in a KC-4 Microplate Reader (BioTek Instruments, Winooski, VT, USA). (+)-Catechin was used for a calibration graph. Total phenolics were expressed as μg of catechin equivalent/g of pepper.

Determination of Antioxidant Activity

DPPH (2,2-Diphenyl-1-picrylhydrazyl)

The DPPH (0.1 mM) radical solution was prepared by dissolving 40 mg DPPH in 1000 ml of MeOH. All the test sample extracts were prepared at a concentration of 5 mg/ml. Aliquots (10 μl) of the extracts were pipetted into a microplate, and the volume of each well was adjusted to 100 μl with MeOH. The DPPH solution (180 μl) was added to all wells, and absorbance was measured at 515 nm for 30 min at a 3 min interval. The DPPH radical scavenging activity was expressed as ascorbic acid equivalents. A standard ascorbic acid solution (0.15, 0.3, 0.45, 0.6, 0.75, 0.9, and 1.05 μg) was used to construct a calibration graph. The radical scavenging activity was expressed by the IC_{50} value (50 % inhibition).

Reducing Power

Different concentrations (0.25, 0.50, 0.75, and 1.00 μg/ml) of the pepper extracts were mixed with sodium phosphate (200 mM) until the volume reached 1.25 ml, and then 1.25 ml of potassium ferricyanide (1 %) was added to the mixture. After incubation at 50 °C in a water bath for 20 min, 1.25 ml of trichloroacetic acid (10 %) was added. The mixture (1 ml) was combined with 1 ml of water and 0.5 ml of ferric chloride (0.1 %). Absorbance was measured at 700 nm against a blank sample and ascorbic acid was used as a standard. In the reducing power assay, antioxidants in extracts reduce ferric chloride and ferricyanide complex to form ferrous complex. This produces a blue colored product with maximum absorbance at 700 nm. The increased absorbance of the reaction mixture indicated stronger reducing power.

Degradation of Deoxyribose

The modified deoxyribose degradation method was used to determine the inhibition of deoxyribose decomposition induced by the hydroxyl radical [25]. Different concentrations (5.00, 2.50, 1.25, and 0.63 mg/ml) of each extract were prepared in phosphate buffer (0.1 M, pH 7.4). All reagents were freshly prepared. Extract (500 μl) was mixed with 200 μl of deoxyribose in buffer (20 mM), 200 μl of ferric chloride ($FeCl_3$, 100 mM): ethylenediaminetetraacetic acid (EDTA, 1 mM) (50:50, v/v), 500 μl of hydrogen peroxide (H_2O_2,10 mM), and 100 μl of ascorbic acid (2 mM). The mixture was incubated at 37 °C in a water bath for 1 h, and then 1 ml of trichloroacetic acid (10 %) and 1 ml of thiobarbituric acid (1 %) in 0.05 M sodium hydroxide was added. The prepared reaction solution was heated at 100 °C for 20 min and cooled to room temperature. Absorbance was measured at 532 nm, and the degradation of deoxyribose was expressed as the inhibition (%).

Statistical Analysis

Data were analyzed using one-way analysis of variance with SAS 9.2 statistical software package (SAS Institute, Cary,

NC, USA). All data are presented as mean±standard deviation of triplicate analyses of each extract. Tukey's comparison of means test was used for significant differences. Pearson correlation coefficients were calculated, and significant differences were determined at the 95 % probability level.

Results and Discussion

Total Phenolics, Carotenoids, and Flavonoids

Four pepper cultivars, including TMH (habanero), TMJ (jalapeno), PA137 (paprika), and B58 (paprika), were extracted using five solvents with different polarities, and the extracts were concentrated, freeze dried, and stored at −20 °C. The highest yield was obtained by MeOH extraction of all pepper cultivars, and the lowest yields were obtained by acetone extraction, with the exception that ethyl acetate provided the lowest yield in paprika peppers (Table 1). The levels of phenolics in various pepper extracts ranged from 36.7 to 73.6 mg of catechin equivalents (CE)/g for TMH, from 27.0 to 39.6 mg of CE/g for TMJ, from 28.5 to 37.2 mg of CE/g for PA137, and from 30.4 to 37.4 mg of CE/g for B58. The levels of total phenolics were the highest in TMH using acetone (Table 1). It is interesting to note that phenolics were extracted not only with methanol and 80 % methanol but also with ethyl acetate and acetone. The levels of phenolics in each extract varied in each pepper. Thondre et al. [26] analyzed total phenolics using different extraction solvents including 70 % acetone, 70 % methanol, and 70 % ethanol, respectively, and Vatai et al. [27] found that phenolic compounds from elderberry and grapes were extracted more in 50 % acetone and 50 % ethanol extraction solvents. When total phenolics were expressed as gallic acid and ferulic acid equivalents, they found that aqueous acetone (1200 μg gallic acid equivalents/g and 1600 μg ferulic acid equivalents/g) had significantly higher yields than other extraction solvents. Additionally, Ornelas-Paz et al. [28] reported that total phenolics were 2307.8 μg of gallic acid equivalents/g in habanero and 2549.7 μg in jalapeno.

The concentrations of carotenoids and flavonoids were measured in pepper extracts (Table 1). The contents of carotenoids measured depended on the type of solvent used to extract peppers. The highest levels of carotenoids were observed in hexane extracts followed by ethyl acetate or acetone. B58 had the highest content of carotenoids (628.8 μg/g), while TMH had the lowest content (47.2 μg/g) in hexane extracts. It has been found that lipophilic (non-polar) solvents were appropriate for extracting carotenoids. For example, Sachindra et al. [29] found that carotenoids were extracted more in shrimp waste using a mixture of isopropyl alcohol and hexane (1:1). The large variance in carotenoid content reflected the fact that paprika-type peppers (PA137 and B58) contained more carotenoids than habanero (TMH) and jalapeño-type (TMJ) cultivars, suggesting that the levels of carotenoids were influenced by genetic variation of peppers [7]. Different levels of flavonoids such as quercetin, luteolin, kaempferol, and apigenin were found in pepper extracts. While flavonoids were not detected in hexane extracts, the remaining four solvents extracted differential levels of flavonoids in all pepper cultivars. The highest levels of flavonoids were extracted in MeOH solvents, and the flavonoids showed approximately a 47-fold difference between the lowest and the highest concentrations. Martins et al. [30] found that the highest contents of total phenolics, total flavonoids, and proteins were obtained using 90 % MeOH in *Larrea tridentata* leaves.

Antioxidant Activity

Different pepper extracts showed variable antioxidant activities due to the selective extraction of bioactive compounds from different pepper cultivars. The antioxidant activity is given as IC_{50} value, which indicates the concentration of extract required to decrease DPPH radical concentration by 50 %. Hexane extracts exhibited the lowest IC_{50} value (0.48–0.74 μg/ml) of radical scavenging activity (Table 2), while MeOH extracts resulted in the highest IC_{50} value (0.84–1.34 μg/ml) in all pepper cultivars. This value was comparable to the IC_{50} (0.15 μg/ml) from ethanol extract of *Capsicum annuum* var. acuminatum [31]. In our results, DPPH free radical was effectively scavenged by pepper extracts from non-polar and mid-polar solvents. It is possible that more carotenoids were extracted in the hexane extract according to data from Table 1. Therefore, DPPH scavenging activity was higher in hexane extract than in other extracts. This result is consistent with a previously reported study [32]. A different study examined the relationship between carotenoids and DPPH radical scavenging activity in mature tomatoes and found that the highest scavenging activity was due to high contents of carotenoids in diethyl ether extraction [33]. However, the use of diethyl ether in routine analysis is not an easy process because ether is highly flammable and easily vaporized. In another study, the contents of carotenoids extracted in hexane were low and DPPH radical scavenging activity was high in bambangan (*Mangifera pajang* Kosterm.) peel [34]. By contrast, Müller et al. [35] reported that DPPH radical scavenging activity was not affected by carotenoids when they were extracted with methanol:tetrahydrafuran mixture in carrot and tomato juice and at relatively low concentrations, while carotenoids strongly influenced other antioxidant activities including Ferric Reducing Antioxidant Power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays.

Table 1 Yields, total phenolics, carotenoids and flavonoids present in solvent extracts of different pepper cultivars

Cultivar	Solvent extraction	Yields (g/100 g of extract)	Total phenolics (mg of catechin equivalents/g of extract)	Carotenoids (μg/g of extract)		Flavonoids (μg/g of extract)
				Capsanthin	β-carotene	
TMH (Habanero)	Hexane	1.3	nd	47.2±0.1[a]	nd	nd
	Ethyl acetate	1.7	45.3±2.1[b]	4.8±0.0[b]	nd	11.9±0.5[b]
	Acetone	0.7	73.6±2.9[a]	nd	nd	6.8±0.1[bc]
	MeOH	33.3	36.7±0.3[c]	nd	nd	24.9±4.2[a]
	MeOH:water (80:20)	5.3	39.8±0.9[c]	nd	nd	23.3±1.0[a]
TMJ (Jalapeno)	Hexane	4.0	nd	nd	103.7±2.2[a]	nd
	Ethyl acetate	2.7	39.6±1.6[a]	nd	21.9±0.2[b]	7.7±0.2[bc]
	Acetone	2.0	34.2±2.3[a]	nd	11.5±0.7[bc]	13.4±0.7[b]
	MeOH	33.7	27.1±0.8[a]	nd	nd	32.4±5.3[a]
	MeOH:water (80:20)	6.3	27.0±1.1[a]	nd	nd	8.3±0.1[bc]
PA137 (Paprika)	Hexane	4.7	nd	nd	354.8±1.8[a]	nd
	Ethyl acetate	2.0	34.1±0.6[a]	nd	3.4±1.4[c]	9.9±0.4[b]
	Acetone	1.3	34.7±1.2[a]	nd	25.0±0.6[b]	2.3±0.1[c]
	MeOH	27.0	37.2±0.3[a]	nd	nd	27.1±1.5[a]
	MeOH:water (80:20)	4.7	28.5±0.9[b]	nd	nd	2.0±0.1[c]
B58 (Paprika)	Hexane	6.7	nd	nd	628.8±1.6[a]	nd
	Ethyl acetate	1.0	33.6±1.9[a]	nd	4.2±0.1[b]	40.6±1.2[b]
	Acetone	1.3	34.9±2.0[a]	nd	7.2±0.1[b]	37.7±0.3[b]
	MeOH	37.0	30.4±1.0[a]	nd	nd	152.2±2.2[a]
	MeOH:water (80:20)	3.7	37.4±0.1[a]	nd	nd	4.7±0.0[c]

Values are means±standard deviation of triplicate analysis; *nd* not detected

The same letter within a column and a pepper cultivar is not significantly different using Tukey's test

Table 2 DPPH radical scavenging activity of various pepper extracts from four cultivars at different concentrations

Cultivar	Solvent extraction	DPPH (IC_{50}) (μg/g)
TMH (Habanero)	Hexane	0.67±0.03
	Ethyl acetate	0.76±0.01
	Acetone	0.71±0.01
	MeOH	0.94±0.01
	MeOH:water (80:20)	0.84±0.03
TMJ (Jalapeno)	Hexane	0.79±0.05
	Ethyl acetate	1.30±0.01
	Acetone	1.33±0.01
	MeOH	1.35±0.01
	MeOH:water (80:20)	1.37±0.04
PA137 (Paprika)	Hexane	0.55±0.02
	Ethyl acetate	0.88±0.01
	Acetone	1.05±0.01
	MeOH	1.03±0.01
	MeOH:water (80:20)	0.95±0.03
B58 (Paprika)	Hexane	0.48±0.01
	Ethyl acetate	0.87±0.01
	Acetone	0.77±0.01
	MeOH	0.97±0.01
	MeOH:water (80:20)	0.96±0.01

Values are means±standard deviation of triplicate analysis

Reducing power was tested at different concentrations for all the pepper extracts (Fig. 1). The reducing power of each extract increased with concentration. Ethyl acetate extracts showed strong reducing power in TMJ and PA137, while acetone extracts had the highest activity in TMH and B58. The results demonstrate that higher reducing power was due to the presence of total phenolics from pepper extracts. Previous studies reported a strong correlation between reducing power and total phenolics in various species of peppers [36–38]. The increase of absorbance at 700 nm in Fig. 1 indicated increased reducing power. In our results, absorbance greater than 2.0 supported the highest reducing power in pepper extracts. Sim and Sil [39] reported that various antioxidant activities in aqueous ethanol extracts of pepper showed similar reducing powers at the concentrations of 500 and 1000 μg/ml.

In the deoxyribose degradation assay, hydroxyl radicals are generated by the presence of hydrogen peroxide, ascorbate, ferric, and ethylenediaminetetraacetic acid. Since deoxyribose is damaged by the radicals and degraded, the inhibition (%) of deoxyribose degradation was measured in the presence of pepper extracts (Fig. 2). MeOH extracts exhibited the maximum scavenging activity, with inhibition values ranging from 47.4 to 52.6 % at 1.6 mg/ml. The lowest inhibition of deoxyribose degradation was observed in MeOH:water (80:20) extracts at all the tested concentrations. In a previous study, various concentrations (0–16.8 mg/ml) of pepper extracts exhibited >95 % inhibition of deoxyribose degradation [40]. These findings indicate that the antioxidant activity of pepper extracts was strongly influenced by the type of

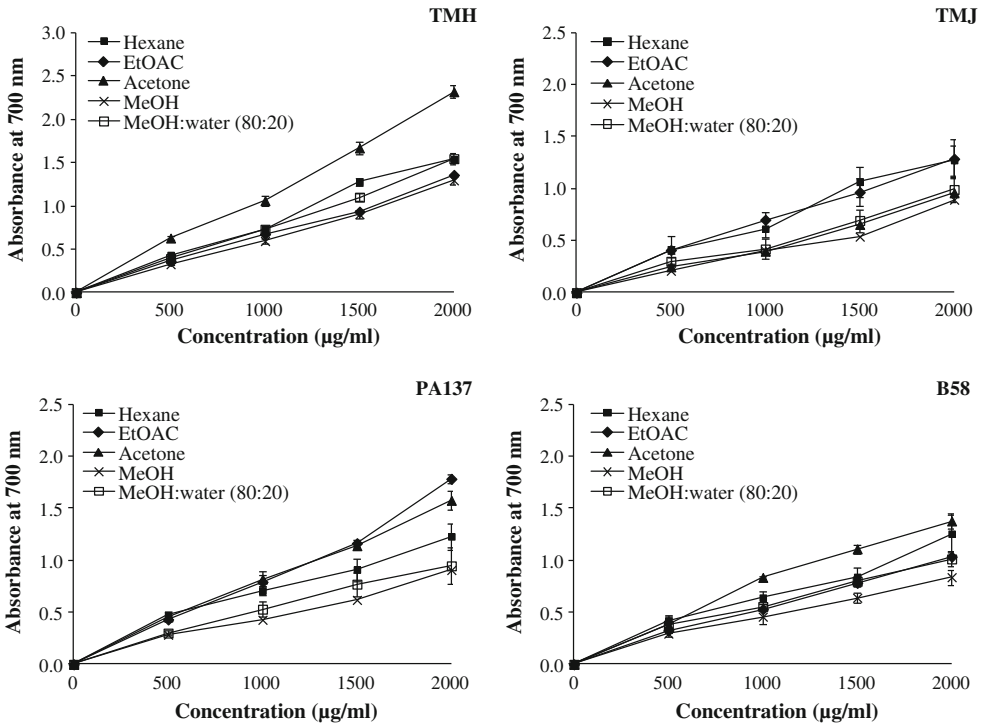

Fig. 1 Reducing power of different pepper extracts from four cultivars at various concentrations. The values are expressed as mean±SD of three independent experiments

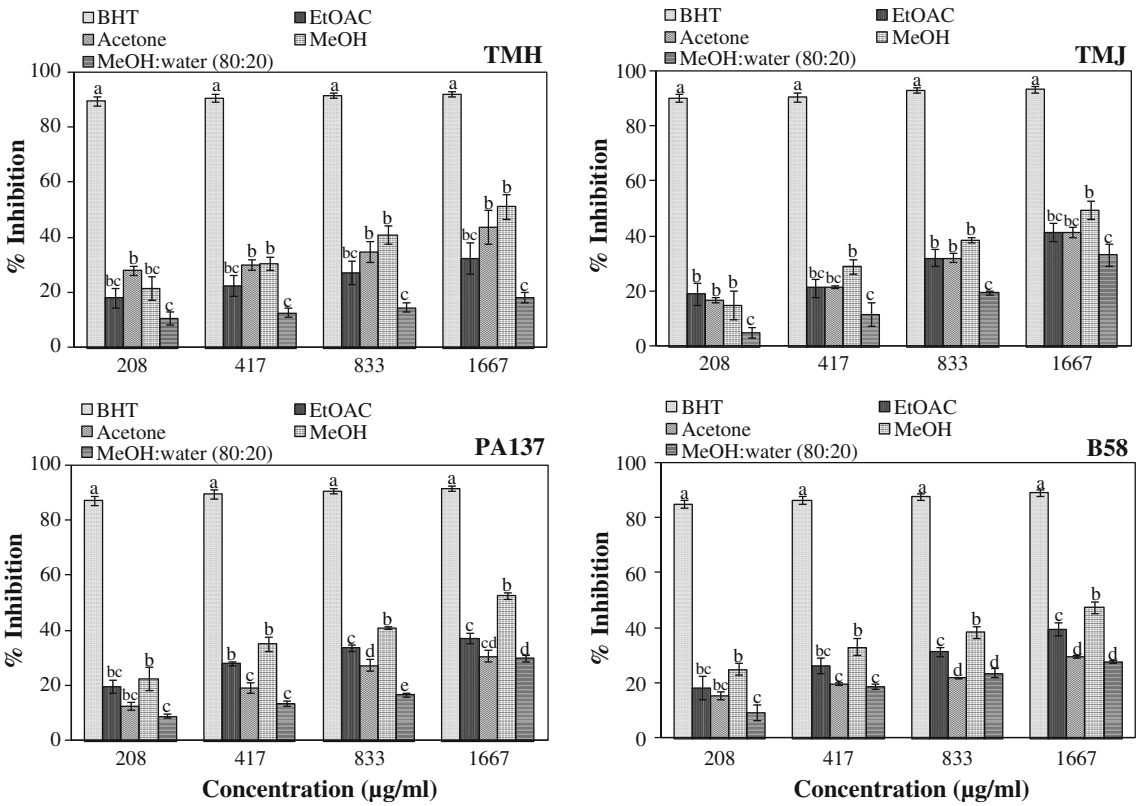

Fig. 2 Inhibition of deoxyribose degradation by various pepper extracts at different concentrations. The values are expressed as mean±SD of three independent experiments

extraction solvents, which selectively extract certain bioactive compounds.

The DPPH radical scavenging activity is one of the most widely used assays to determine antioxidant activity of peppers. Reducing power has been tested to measure the reducing ability of antioxidants in specific concentrations of hydrophilic and lipophilic samples. In a deoxyribose degradation assay, hydroxyl radicals will attack the deoxyribose to degrade a series of fragments, some or all of which react with thiobarbituric acid (TBA) to get pink chromogen after heating. The degree of color formation depends on antioxidants present in the pepper samples. Therefore, it is important to establish the relationship between solvent properties and antioxidant activity of bioactive compounds.

Correlations

Bioactive compounds from pepper extracts were found to have deferential antioxidant activity in different pepper cultivars. The DPPH results showed a positive correlation with total phenolics (r=0.66), carotenoids (r=0.79), and flavonoids (r=0.85). In addition, reducing power and inhibition of deoxyribose degradation was also significantly correlated to flavonoids (r=0.77 and 0.83) and total phenolics (r=0.72 and 0.81). Interestingly, the levels of carotenoids were not correlated with reducing power activity or inhibition of deoxyribose degradation. This may be due to the fact that lipophilic carotenoids did not react effectively in hydrophilic assays such as the reducing power and deoxyribose inhibition assays. The results of the present study are supported by other studies. For example, Serrano et al. [41] demonstrated that the hydrophilic antioxidant activity was strongly correlated to total phenolics, and lipophilic antioxidant activity was highly correlated to carotenoids in sweet peppers. Xu and Chang [42], also concluded that in order to verify different compositions of antioxidants in legume foods, comparison of various extraction solvents was necessary for better extraction [42].

Conclusions

In this study, antioxidant activities and bioactive compounds were compared among pepper extracts from different extraction solvents. The concentrations of total phenolics, carotenoids, and flavonoids were highly dependent on the nature of the solvent used to extract the compounds from peppers. Hexane and methanol were the most efficient

solvents in extracting carotenoids and flavonoids, respectively. Hexane extracts showed strong 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, and ethyl acetate and acetone extracts showed the highest reducing power in different pepper cultivars. For the deoxyribose degradation assay, methanolic pepper extracts showed the highest inhibition. These observations demonstrated that solvent properties, with different polarity, can significantly increase the extraction efficiency of specific lipophilic or hydrophilic compounds in different peppers as well as antioxidant activity.

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