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ANTIOXIDANT ACTIVITY OF SPICE EXTRACTS AND PHENOLICS IN COMPARISON TO SYNTHETIC ANTIOXIDANTS

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ABSTRACT

The antioxidant capacities of 30 spices used in ready meals and a selection of key compounds from spices were investigated in the current study using ferric reducing antioxidant properties (FRAP), 2,2'-azinobis(3-ethylebenzothiaziline-6-sulfonate) (ABTS) and microsomal lipid peroxidation (MLP) assays. Antioxidant capacities of the spice extracts were compared to 5 popular synthetic antioxidants [butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylated hydroquinone (TBHQ), propyl gallate (PG) and octyl gallate (OG)]. Clove extracts had the highest antioxidant capacities as measured by FRAP, ABTS and MLP. Extracts from garlic powder were the lowest ranked of all the spices examined. Synthetic antioxidants were ranked in the following decreasing order of antioxidant activity PG > BHA > TBHQ > OG > BHT. Rosmarinic acid, a polyphenol commonly found in lamiaceae spices and eugenol from clove had higher antioxidant capacities than that of all synthetic antioxidants investigated. Antioxidant capacities of kaempferol from apiaceae spices, capsaicin from chilli, curcumin from turmeric, thymol from thyme and gingerol from ginger were also comparable to most of the synthetic antioxidants.

***Key words**: Antioxidants, spices, phenolics, assay, rosmarinic acid, eugenol*

INTRODUCTION

Oxidative deterioration of food products during processing and storage produces off-flavour which affect their marketability. Furthermore, the compounds such as aldehydes, ketones and organic acids produced through oxidation process have been impicated in cardiovascular diseases, mutagenesis and carcinogenesis[1]. In the past synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylated hydroquinone (TBHQ), propyl gallate (PG) and octyl gallate (OG) have been used extensively to inhibit oxidation in foods. However in recent times epidemiological studies have pointed to the possible health risks associated with consumption of synthetic antioxidants[1] and strict regulations now govern their use in foods[2]. Consumers are also demanding foods which are more 'fresh like' in appearance and this has resulted in a demand for antioxidants derived from natural sources. Spices are abundant sources of polyphenolic compounds which have strong antioxidant capacities[3] and could potentially replace the synthetic antioxidants in food systems and offer additional health benefits. Consumption of spices has been implicated in the prevention cardiovascular diseases, carcinogenesis, inflammation, atherosclerosis[4]. This is primarily due to presence of polyphenols including rosmarinic acid in lamiaceae spices, eugenol in clove and pimento, curcumin in turmeric, capsaicin in chilli, kaempferol cumin and fennel, gingerol in ginger, caffeic acid in thyme and fennel[3,5]. Spices also have antimicrobial properties which can help extend the shelf-life of foods. Moreover consumer acceptance towards spices or spice principles is appreciably high[6]. The aim of the present study was to evaluate the antioxidant properties of spice extracts and some key compounds derived from spices using three in-vitro antioxidant capacity assays namely the ferric reducing antioxidant properties (FRAP), 2,2'-azinobis(3-ethylebenzothiaziline-6-sulfonate)

(ABTS) and microsomal lipid peroxidation (MLP) assays. In order to evaluate the technological and biological potential of the spices, values from these assays were compared to those 5 widely used synthetic antioxidants.

EXPERIMENTAL

Materials

Dried and ground Clove, Cinnamon, Pimento, Rosemary, Oregano, Marjoram, Bay, Sage, Thyme, Basil, French onion, Coriander, Cumin, Fennel, Onion, Cayenne pepper, Chilli, Turmeric, Celery, Mustard, Paprika, Black pepper, White pepper, Nutmeg, Mace, Cardamom, Garlic, Parsley, Ginger and Aniseed which were provided by AllinAll Ingredients Ltd., (Dublin 12, Ireland).

Chemicals

Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylated hydroquinone (TBHQ), propyl gallate (PG) and octyl gallate (OG), rosmarinic acid (RA), eugenol, capsaicin, curcumin, 6-gingerol, kaempferol, ferulic acid, thymol, microsomes pooled from female rat (Sprague Dawley) liver were purchased from Sigma-Aldrich, USA.

Methods

Preparation of spice extracts

Dried and ground samples (1g) were homogenised for 1 min at 24,000 rpm using an Ultra-Turrax T-25 Tissue homogenizer (Janke & Kunkel, IKA®-Labortechnik, Saufen, Germany) in 25 mL of 80% methanol at room temperature (~23 °C). The

homogenised extract was shaken overnight at 1500 rpm. The extract was then centrifuged at 3000 rpm for 15 min and filtered through 0.22 µm polytetrafluoethylene (PTFE) filters.

Ferric ion reducing antioxidant power (FRAP) assay

The FRAP assay was carried out as described by Stratil and others[7] with slight modifications. The FRAP reagent was made fresh before each experiment. The FRAP reagent was prepared by mixing 38 mM sodium acetate anhydrous in distilled water pH 3.6, 20 mM $FeCl_3.6H_2O$ in distilled water and 10 mM 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl in a proportion of 10:1:1. To each sample 100 µL of appropriately diluted sample extract and 900 µL of FRAP reagent was added and incubated at 37 °C for 40 min in the dark. In the case of the blank 100 µL of methanol was added to 900 µL of FRAP reagent. The absorbance of the resulting solution was measured at 593 nm by spectrophotometer. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid) (a synthetic antioxidant) at concentrations from 0.1 mM-0.4 mM was used as a reference antioxidant standard. FRAP values were expressed as g Trolox/100 g DW of the sample.

The 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay

The ABTS assay was carried out according to the method of Miller and others[8] with slight adjustments. The principal reagents were phosphate buffered saline (80 mM/L, pH 7.4), chromogen, and hydrogen peroxide (250 µM/L). The chromogen contained metmyoglobin (6.1 µM/L) and ABTS (610 µM/L). The phosphate buffered saline was mixed with chromogen and hydrogen peroxide to give –final concentrations as outlined above. For each sample 20 µL of the appropriately diluted sample extract

was added to 1 mL of the chromogen and incubated at 37 °C and the initial absorbance recorded. 200 µL of the hydrogen peroxide was added to the mixture, incubated at 37 °C in the dark and the final absorbance was measured exactly after 3 min. Initial absorbances were deducted from the final absorbance to get the Δ absorbance. This value was then used to calculate antioxidant capacities as compared to the synthetic antioxidant Trolox (0.1 mM -0.4 mM) as outlined for the FRAP assay.

Microsomal lipid peroxidation (MLP) assay

The microsomal lipid peroxidation assay was carried out as outlined by van der Sluis and others[9] with slight modifications. Briefly rat liver microsomes (Sigma-Aldrich, 20 mg protein/1 mL) were thawed on ice and diluted 10 fold with Tris-HCl buffer (50 mM, pH 7.4) containing KCl (150 mM). The mixture was then vortexed and sonicated for 3 min to obtain a homogenous solution. 125 µL of this solution was aliquoted into an eppendorf tube and centrifuged at 10, 000 rpm for 30 min. After centrifugation the supernatant was removed and the pellets were re-suspended as uniformly as possible in 440 µL of Tris-HCl buffer (50 mM, pH 7.4). This was achieved by micro-pippetting in and out of the eppendorf tubes and vortexing followed by sonication for 1 min. Aliquots (30µL) of appropriately diluted samples were added to the microsomal solution and vortexed well. Lipid peroxidation was induced by adding 15 µL of 4 mM ascorbic acid and 15 µL of 0.2 mM $FeSO_4$. The mixture was vortexed again to mixed well. Eppendorf tubes were incubated at 37 °C for 1 hour. The reaction was stopped by adding 500 µL of 0.83 % thiobarbituric acid in TCA-HCl (16.8 % w/v trichloroacetic acid in 0.125 N HCl). Thiobarbituric acid reactive species produced as a result of lipid peroxidation were measured after heating the eppendorf tubes at 80 °C for 15 min. The mixture was centrifuged at 10, 000 rpm for 3 min and

the absorbance of the pink coloured supernatant was measured at 540 nm. The absorbance of the blank solutions (440 µL of Tris-HCl buffer 50 mM, pH 7.4) without microsomes was measured at the same wavelength. In case of control, 30 µL methanol was used instead of sample extract. The concentration of extract/pure compound required to cause a 50% reduction in the absorbance of the control was calculated (IC_{50}). For ease of interpretation IC_{50} was converted to anti-radical powers ($1/ IC_{50}$) as this value is directly proportional to antioxidant capacity. Three replicates for both samples and standard were performed in each of the two batches of the experiment.

RESULTS AND DISCUSSION

Antioxidant capacity of spice extracts as measured by FRAP, ABTS and MLP assays

Clove extracts had the highest TEAC (Trolox Equivalent Antioxidant Capacity) value as measured by the ABTS assay followed by cinnamon (Table 1). This was in agreement with the finding of Shan and others[3]. Clove also had highest antioxidant capacity as measured in FRAP and MLP assays (Table 1). The antioxidant potential of clove extracts may be due to its strong hydrogen-donating and metal chelating ability, as well as it's effectiveness as a scavenger of hydrogen peroxide, superoxide and free radicals.. In general, the spices of Myrtaceae family (clove and pimento), Lauraceae family (cinnamon and bay) and lamiaceae family (rosemary, oregano, marjoram, sage and thyme) had very high TEAC values (Table 1). This observation was also true for the FRAP assay where antioxidant capacities of all these spice extracts were higher than mean values (7.91 g Trolox/100 g DW). The mean ARP

value for all spice extracts in the MLP assay was 1.68 $(g/L)^{-1}$. In agreement with results from the FRAP and ABTS assays ARP values for clove, pimento, cinnamon, bay leaf, rosemary, oregano, marjoram, sage were higher than mean values. Basil extracts had the lowest antioxidant capacity among the Lamiaceae spices in all the assays tested. The high antioxidant capacity of Myrtaceae, Lauaraceae and Lamiaceae spices is well known[3,10,11] in particular for Lamiaceae spices. Rosemary extracts had the highest antioxidant capacity as measured by the ABTS assay among the Lamiaceae spices, whereas in the FRAP assay oregano had a stronger antioxidant activity than rosemary. Interestingly in the MLP assay sage extracts had the highest antioxidant capacity among the Lamiaceae spices. The principal polyphenolic compound present in spices of Myrtaceae family is eugenol a compound with a strong antioxidant potential. Lauracae spices contain eugenol which might be responsible for their higher antioxidant activity. The strong antioxidant activity of cinnamon might be attributed to its high cinnamaldehyde content in addition to eugenol. The key antioxidant compound in Lamiaceae spices is rosmarinic acid[3]. Extracts from white pepper of Piperaceae family and cardamom of Zingiberaceae family had low antioxidant capacities. Among all the extracts examined garlic powder extract had the lowest antioxidant capacity in all assays. In fact, the antioxidant capacity of garlic was 171 times lower than that of the clove highest ranked as per FRAP assay.

Highly significant correlations ($p<0.05$) between radical scavenging activities as measured using the FRAP, ABTS and MLP assays were observed ($R^2= 0.813$ for FRAP vs ABTS (Figure 2), $R^2= 0.697$ for MLP vs FRAP (Figure 3) and $R^2= 0.639$ for MLP vs ABTS (Figure 4)). The correlation co-efficients of MLP vs FRAP and MLP vs ABTS was significantly ($p<0.05$) lower than that of the FRAP vs ABTS due to the fact that sage exhibited exceptionally higher ARP value in MLP assay. When sage

ARP value was excluded from the calculation, the correlation co-efficient (R^2) between MLP vs FRAP and MLP vs ABTS was 0.9527 and 0.778 respectively.

Antioxidant capacity of pure spice phenolics in comparison to synthetic antioxidants as measured by FRAP, ABTS and MLP assays

Among the pure compounds tested rosmarinic acid had the highest antioxidant capacity followed by eugenol in all the methods applied. Rosmarinic acid had an antioxidant capacity twice as high as that of PG the strongest synthetic antioxidant as per the FRAP and MLP assays. Eugenol also had a higher antioxidant activity than that of PG. The strong antioxidant potential of rosmarininc acid is not surprising since it possesses four phenolic groups capable of stabilising free radicals[12,13]. The strong antioxidant potential of eugenol may be related to the position of the single hydroxyl group on the phenol group. The antioxidant capacities of kaempferol, ferulic acid, 6-gingerol and curcumin as measured by FRAP and MLP assays were higher than BHT the most widely used synthetic antioxidant in food systems. These results suggest that the spice phenolics especially rosmarinic acid and eugenol could potentially be used in food systems in order to prevent oxidative deterioration of foods. In fact the antioxidant capacity of clove extract as measured by the FRAP assay (61.63 g Trolox/100 g DW) was close the antioxidant capacity of BHT (80.85 g Trolox/100 g DW). Antioxidant capacities of extracts from cinnamon, pimento, rosemary, oregano, sage and marjoram were 4-5 times lower than that of BHT. The ranking of the pure natural phenolics in terms of antioxidant capacity as measured by both FRAP and MLP assay followed the following decresasing order: rosmarinic acid > eugenol > kaempferol > ferulic acid > gingerol > curcumin > thymol > capsaicin (range: 406.29-17.35 g Trolox/100 g DW in FRAP assay and 175.24-20.05 $(g/L)^{-1}$ in MLP assay)

(Figure1). The ABTS assay followed a slightly different order which was: rosmarinic acid > eugenol > kaempferol > ferulic acid > gingerol > curcumin > capsaicin> thymol (range: 704.47-8.38 g Trolox/100 g DW).

CONCLUSION

Spice phenolics having very high antoxidant capacity could potentially substitute the synthetic antioxidants in foods to prevent oxidative deterioration. Rosmarinic acid and eugenol had significantly higher antioxidant capacity than that of PG ($p<0.05$), the strongest synthetic antioxidant. Extracts from spices of the Myrtaceae, Lauraceae and Lamiaceae families might also be used in place of synthetic antioxidants. The antioxidant capacity of both spice extracts and pure compounds as measured by FRAP, ABTS and MLP followed the same trend. The high correlation coefficients among three different assays indicated that the antioxidant capacity of spice samples could be predicated from one assay to other.

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Table 1. Antioxidant capacity (AC) of spice extracts as measured using the ABTS, FRAP and MLP assays AC's are ranked in descending order as per FRAP assay.

Spices	Family	FRAP	ABTS	MLP
		g Trolox/100 g DW		ARP $(g/L)^{-1}$
Clove	Myrtaceae	61.63 ± 0.776	33.36 ± 0.218	10.48 ± 0.350
Cinnamon	Lauraceae	24.27 ± 0.102	20.78 ± 0.176	4.00 ± 0.061
Pimento	Myrtaceae	20.54 ± 0.365	20.56 ± 0.104	3.84 ± 0.030
Oregano	Lamiaceae	18.86 ± 0.106	18.09 ± 0.099	2.26 ± 0.016
Rosemary	Lamiaceae	14.54 ± 0.250	18.34 ± 0.198	3.05 ± 0.031
Sage	Lamiaceae	14.28 ± 0.261	14.79 ± 0.344	9.82 ± 0.296
Marjoram	Lamiaceae	12.26 ± 0.025	8.14 ± 0.169	2.47 ± 0.041
Mace	Myristicaceae	9.82 ± 0.812	2.70 ± 0.022	0.82 ± 0.014
Thyme	Lamiaceae	8.80 ± 0.018	15.31 ± 0.100	1.48 ± 0.017
Bay	Lauraceae	8.54 ± 0.440	17.55 ± 0.292	2.28 ± 0.048
Basil	Lamiaceae	5.83 ± 0.076	2.87 ±0.026	1.59 ± 0.007
French onion	N/A	4.86 ± 0.058	2.86 ± 0.029	1.13 ± 0.186
Ginger	Zingiberaceae	4.36 ± 0.086	1.96 ± 0.035	0.75 ± 0.004
Nutmeg	Myristicaceae	4.31 ± 0.012	2.16 ± 0.027	0.76 ± 0.001
Turmeric	Zingiberaceae	2.75 ± 0.040	2.05 ± 0.020	1.03 ± 0.014
Celery	Apiaceae	2.29 ± 0.129	1.84 ± 0.030	1.22 ± 0.006
Black pepper	Piperaceae	2.13 ± 0.052	2.23± 0.017	0.68 ± 0.012
Cayenne pepper	Solanaceae	1.92 ± 0.014	1.74 ± 0.019	0.60 ± 0.003
Mustard	Brassicaceae	1.85 ± 0.029	0.68 ± 0.169	0.29 ± 0.002
Cumin	Apiaceae	1.83 ± 0.010	1.19 ± 0.009	0.63 ± 0.004
Paprika	Solanaceae	1.68 ± 0.004	1.22 ± 0.016	0.35 ± 0.005
Chilli	Solanaceae	1.63 ± 0.169	1.50 ± 0.019	0.34 ± 0.002
Aniseed	Apiaceae	1.62 ± 0.004	1.29 ± 0.010	0.63 ± 0.187
Fennel	Apiaceae	1.52 ± 0.001	1.23 ± 0.017	0.37 ± 0.004
Parsley	Apiaceae	1.28 ± 0.002	1.35 ± 0.019	0.28 ± 0.001
White pepper	Piperaceae	1.19 ± 0.007	1.33 ± 0.037	0.34 ± 0.002
Coriander	Apiaceae	1.13 ± 0.024	1.27 ± 0.009	0.32 ± 0.003
Cardamom	Zingiberaceae	0.59 ± 0.004	0.20 ± 0.006	0.28 ± 0.001
Onion	Alliaceae	0.43 ± 0.020	0.19 ± 0.002	0.29 ± 0.001
Garlic	Alliaceae	0.36 ± 0.006	0.18 ± 0.002	0.06 ± 0.001

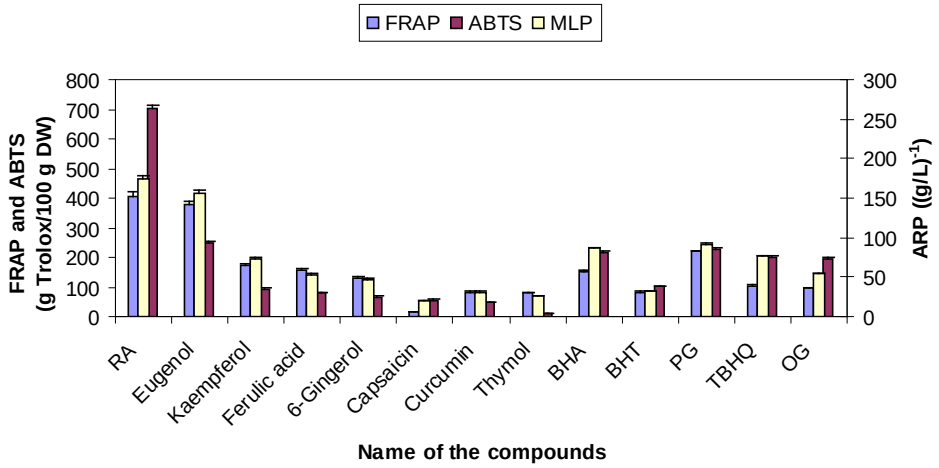

Figure 1. Antioxidant capacity (AC) of spice phenolics and synthetic antioxidants as measured using the ABTS, FRAP and MLP assays

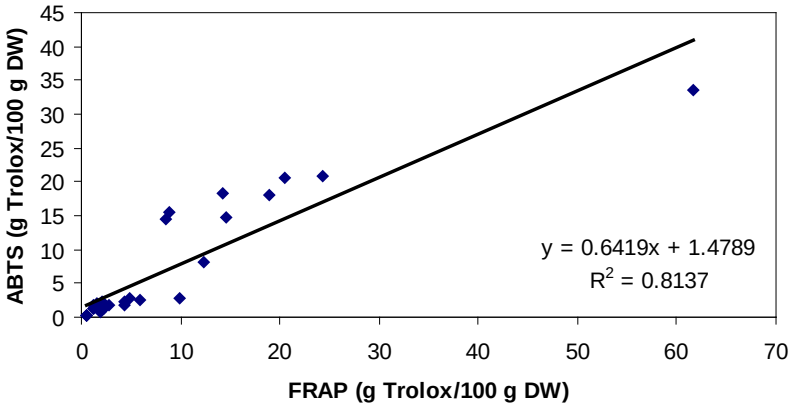

Figure 2. Relationship between the antioxidant capacities as measured by ABTS and FRAP assay of methanolic extracts from 30 spices

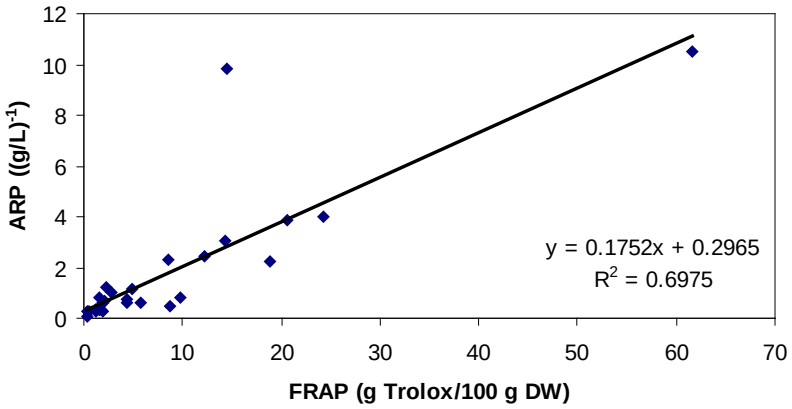

Figure 3. Relationship between the antioxidant capacities as measured by MLP and FRAP assay of methanolic extracts from 30 spices

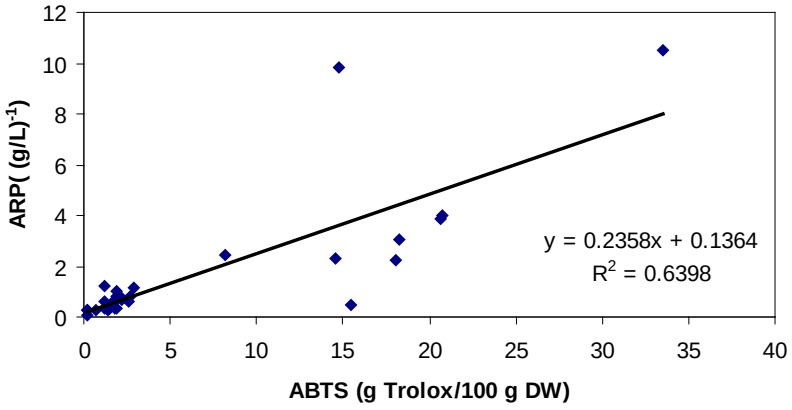

Figure 4. Relationship between the antioxidant capacities as measured by MLP and ABTS assay of methanolic extracts from 30 spices