SHORT COMMUNICATIOS

Comparative Study of Different Spices and Herbs Total Antioxidant Capacity by Employing Ferric Reducing Antioxidant Power (FRAP) Assay

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ABSTRACT

Natural antioxidants extracted from spices and herbs are reported to be effective in inhibiting oxidation due to the anti-oxidant compounds they contain. However the use of these natural antioxidants are limited mainly due to lack of proven evidence of the potential they have. A comparative study on the total antioxidant capacity of 12 spices and herbs collected in the market places of Ethiopia and Belgium was analyzed using FRAP assay .The study demonstrated that there is more than 1000-fold difference among total antioxidant capacity between spices and herbs. Among the studied spices and herbs, the highest antioxidant capacity was found in clove followed by cinnamon and rosemary. Furthermore a two-fold variation in FRAP value was observed between the same spice of different origin.

Key words: Oxidation; Spices and herbs; Antioxidant; FRAP assay

INTRODUCTION

Antioxidants may be defined as 'any substance when present at low concentration, compared with those of the oxidizable substrates significantly delay or prevent the oxidation of the substrate (Shahidi, 2000; Antolovich *et al.*, 2002). Antioxidants are of interest to both food scientists and health professionals. They are often added to foods to stabilize them and prevent off-flavor development. Considerable interest has been also expressed for their potential role as therapeutic agents (Shahidi, 2000). Because many of the naturally-occurring antioxidants are destroyed during manufacturing processes there is a need to extend the shelf life of the product including those made from muscle particularly cooked meat that develop off flavor faster than uncooked counterpart (Güntensperger *et al.*, 1998 ; Jayathilakan *et al.*,2007a ; Jayathilakan *et al.*,2007b).

Cho *et al*. (2004) and McBride *et al*. (2007) suggested that lipid oxidation and deterioration in appearance and microbial growth in food products can be controlled minimized or delayed using antioxidants. In order to use any antioxidant in food, it must be safe, easy to incorporate, effective at low concentrations, with no undesirable odor, flavor or colour, heat stable, nonvolatile and with good carry through properties and cost-effective (McBride *et al*., 2007).

Antioxidants used in food system may be either synthetic such as Butylated hydroxyanisole (BHA) or Butylated hydroxytoluene (BHT) or natural food additives. However due to the growing awareness on the potential health hazard caused by synthetic antioxidants and their well known toxicity effects there is a renewed interest in use of naturally occurring antioxidants originated from plants (especially spices and herbs) (Antolovich *et al.*, 2002; Yu *et al*., 2002; Jayathilakan *et al.*, 2007a). This trend is also reflected in the recently EU Directive 2006/52/EC referring to the necessity of reduction in use of synthetic antioxidants such as nitrites (European Union, 2006).

Natural antioxidants are primarily plant phenolics with multifunctional effects and can act as reducing agents, free radical terminators during the initiation or propagation step of oxidation reaction, metal chelators and singlet oxygen quenchers (Jayathilakan *et al*., 2007b). However the use of these natural antioxidants are limited mainly due to lack of proven evidence of the potential they have and the imposition of undesirable flavours and odors (Jayathilakan *et al.*,2007a ; Tang *et al.*,2000; McBride *et al*.,2007).

Prediction of antioxidant activity is extremely difficult and demands the empirical evidence derived from shelf-life stability trials specific to each food model systems or measuring the total antioxidant capacity of biological samples using available methods such as FRAP assay, which depends upon the reduction of ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH (McBride *et al*., 2007 ; Szôllôsi and Varga ,2002). Thus, this research therefore was conducted to quantify and compare the total antioxidant potential of some commonly used spices and herbs by employing FRAP assay.

MATERIALS AND METHODS

Sample preparation

A total of 12 commercially available spices and herbs were collected from market places in Belgium and Ethiopia (Table 1). They were identified and classified according to standard botanic nomenclature. Spices and herbs used for the study were: Rosemary extract and powder (*Rosmarinus officinalis*), two types of ginger (Ginger from Belgium and Ethiopia) (*Zingiber officinale*), Onion (*Allium cepa*), White pepper (*Piper nigrum*), Cardamom (*Elettaria cardamomum*), Nutmeg (*Myristica sp*.), Clove (*Syzygium aromaticum*), Garlic (*Allium sativum*), Korarima (*Aframomum*

korarima), Turmeric (*Curcuma longa*), Cinnamon (*Cinnamomum verum*) and Mustard (*Brassica oleracea*). All they were weighed to 0.05g and 6 ml water/methanol (1:6 v/v) extraction solvent was added. The mixture was shaken on ultrasonic bath for 15 min. The extracts were centrifuged at 1000 rpm for 5 min and the decanted supernatant was ready for analysis after appropriate dilution with demineralized water (50:50 v/v).

Analysis and measurements

The FRAP assay was performed as per the method describe by Benzie and Strain (1996). The protocols used for the preparation of the reagents were: Acetate buffer, 300 mmol/l pH 3.6 (3.1g sodium acetate x $3H_2O$ and 16 ml concentration acetic acid per 1ml of buffer solution), 10 mmol/1,2,4,6-tripyidyl-s-triazine (TPTZ) in 40 mmol/1 HCl and20 mmol/l $FeCl_3$ x $6H_2O$ in demineralized water. FRAP working solution was made by mixing 25ml of acetate buffer , 2.5ml TPTZ solution and 2.5 ml $FeCl_3$ x $6H_2O$ solution. The working solution was freshly prepared.

Aqueous solution of known Fe (II) concentration was used for calibration (in the range of 100-1000 µmol/l). One hundred micro liter of diluted sample was transferred to a 1 cm cuvette (cell) each time in duplicate then added 300µl demineralized water and finally 3 ml of freshly prepared FRAP working solution was added . The cuvettes were transferred to the spectrophotometer connected with water bath at 25 °C. The measurement was started with a blank sample in parallel and monitoring up to 20 min at 593nm. In the FRAP assay, reductants ("antioxidants") in the sample reduce Fe (III) tripyridyltriazine complex, present to the blue ferrous form, with an increase in absorbance at 593 nm. Change in absorbance is proportional to the combined (total) ferric reducing antioxidant power (FRAP value) of the antioxidant in the sample. Calculations was done using the calibration curve and the tested spices and herbs antioxidant capacity was compared and ranked based on their FRAP values (mmol Fe_2+/g DW).

Data handling and statistical analysis

FRAP assay was performed for 12 spice and herbs in duplicate and data was submitted to General Linear Model (GLM) procedure from SPSS 15.0 (SPSS, 2006) statistical software package to determine the total antioxidant capacity. Summary statistics mean FRAP (mmol Fe_2+/g DW) value and standard deviation were computed the antioxidant sources were ranked. The treatments whenever found significant, the tukey test was used for pair wise comparisons among the different treatments at the 5% ($p < 0.05$) significant level.

RESULTS AND DISCUSSION

Tested spices and herbs showed significant ($p<0.05$) difference in their antioxidant capacity. The ranking order of the mean FRAP values were summarized in Table 1. Among the dried spices and herbs clove showed higher level of antioxidant capacity (1646.7 mmol Fe^{2+}/g DW) followed by cinnamon, rosemary and garlic with FRAP value between 113.6 and 605.9 mmol Fe^{2+}/g DW. Cardamom, Onion, White pepper, Korarima and Ginger (from Ethiopia) revealed intermediate FRAP value (between 50 and 99 mmol Fe^{2+}/ g DW). Mustard, Turmeric, Ginger (From Belgium) and Nutmeg all showed lower FRAP value (< 50 mmol Fe^{2+}/g DW).

Clove, Cinnamon and Rosemary revealed significant different FRAP values with other spices and herbs, where as there was no significant ($p>0.05$) difference among the remaining spices and herbs.

Dragland *et al*. (2003) reported more or less similar ranks among individual spices and herbs, although their relative values are different and not easily compared to the values in this study that may indicate the repeatability of the

FRAP assay. The authors suggested comparison of values obtained from the assay with data in the literature was problematic due to large variability within the spices and herbs. Comparison with previous work was further complicated because of the difference in agro ecology zones the spices and herbs grown (no information available on collection time, variety information and the different extraction methods used in different studies (lack of standardization of the assay).

The present study revealed a two–fold variation between the same spices collected from different regions. This may be due to the fact that the effectiveness and composition of plant metabolites is affected by climate, geographical location, and season and growth condition (Prance, 1994 as cited in Buwa and Staden 2007).

The study also observed that (Table 1)there is more than 1000-fold difference in total antioxidant capacity among studied spices and herbs.

Though the FRAP assay was used for human cases originally (Benzie and Strain 1996), similar ranking of spices and herbs used in the present study with previous studies we found FRAP method is appropriate to measure the total antioxidant capacity and state of biological materials such as spices and herbs.

CONCLUSION

These natural antioxidants have better consumer acceptance and can be used to replace synthetic antioxidants in commercial meat products. However prediction of antioxidant activity is extremely difficult and demands the empirical evidence derived from shelf-life stability trials specific to each food model systems (McBride *et al*., 2007). Nissen *et al*. (2004) also suggest the effect of a certain potential antioxidant might vary considerably depending on a complex interaction between various factors, type and concentration of active compound(s) and nature of the food systems .Thus the extracts should always be tested in the actual product as the anti-oxidative activity may deviate significantly between model systems.

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Table 1. Mean Ferric reducing antioxidant power (FRAP) and ranking order of Studied spices and herbs

Spices and herbs	Origin	FRAP (mmol Fe^{2+} /g DW[1])	Rank
Cardamom	Belgium	99.25^{de}	6
Cinnamon	Ethiopia	605.9^{b}	2
Clove	Ethiopia	1646.7^{a}	1
Garlic	Ethiopia	113.6^{de}	5
Ginger (BE)	Belgium	25.10^{e}	13
Ginger (ET)	Ethiopia	50.22^{de}	10
Korarima	Ethiopia	51.21^{de}	9
Nutmeg	Belgium	13.0^{e}	14
Mustard	Ethiopa	32.83^{de}	11
Onion	Belgium	97.80^{de}	7
Rosemary	Ethiopia	273.98^{c}	3
Turmeric	Ethiopia	29.37^{de}	12
White pepper	Belgium	79.10^{de}	8

Means followed by the same superscript letter within each column are not significantly different at $P = 0.05$

1= Dry weight, BE= Belgium , ET= Ethiopia

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