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Amit Jaiswal
Technological University Dublin, amit.jaiswal@dit.ie

Gaurav Rajauria
Dublin Institute of Technology

Nissreen Abu-ghannam
Dublin Institute of Technology

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Bioactive Potential of Marine and Terrestrial Vegetables: A Comparative Study

A.K. Jaiswal, G. Rajauria and N. Abu-Ghannam

School of Food Science and Environmental Health, College of Sciences and Health, Dublin Institute of Technology, Cathal Brugha Street, Dublin 1, Ireland. Email: amit.kumar@dit.ie)

ABSTRACT

Polyphenols are extensively used in food, beverage, pharmaceuticals and nutraceutical industry for their positive effects on human health. Present study was designed to estimate the differences in polyphenols level and antioxidant capacity between marine and terrestrial vegetables. Considerable variations in the total phenolic content (TPC) and total flavonoid content (TFC) were observed, which ranges from 20.4 to 140.2 mg GAE/g of extracts (dw) for TPC and 15.4 to 50.4 mg QE/g of extracts (dw) for TFC. Furthermore, antioxidant capacity was confirmed by all the extracts. Results showed that marine vegetables had significantly higher polyphenols content and antioxidant property compared to terrestrial counterparts.

Keywords: Polyphenols, antioxidant capacity, terrestrial vegetables, marine vegetables

INTRODUCTION

Free radicals such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous factors and exogenously result in extensive oxidative stress. Oxidative stress is implicated in a wide variety of diseases, including the development of various human chronic diseases such as cardiovascular disease, certain cancers, and a number of neurodegenerative diseases (Aruoma, 1998). Furthermore, problem of oxidation is most common aspects of food preservation, especially when the products develop undesirable flavours, unpleasant taste, rancid odours, discoloration and other forms of spoilage often responsible for the loss of quality, and safety and shortening of shelf life. All aerobic organisms have antioxidant defences that protect against oxidative damages, and numerous damage removal and repair enzymes are present to remove or repair damaged molecules (Ali et al. 2001). However, this natural antioxidant mechanism can be inefficient; hence, dietary intake of antioxidants becomes important (Halliwell 1994). Besides playing an important role in physiological systems, antioxidants have been applied in the food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats. Synthetic antioxidants such as butylated hydroxy toluene (BHT) and butylated hydroxyanisole (BHA) have been commonly used in the food industry to prevent oxidation of food. It has been reported that these compounds are associated with some side effects (Botterweck et al. 2000). Natural antioxidants are of high interest as alternatives for synthetic antioxidants to prevent oxidation in food systems. Several studies have focused on natural antioxidants from marine (Ganesan et al., 2008; Rajauria et al., 2010; Keyrouz et al., 2011; O'Sullivan et al., 2011) and terrestrial vegetables (Jacob et al., 2011; Jaiswal et al., 2012) and their application in food systems to prevent oxidation. In the present study, bioactive potential of vegetables from marine and terrestrial origin were evaluated, and a comparative study was carried out between these vegetables.

MATERIALS AND METHODS

Extracts preparation: Extraction of phenolic compounds was carried out according to the method of Ganesan et al., 2008. Briefly, 5 g of crushed vegetable samples were added to three different flasks and extracted using 60% methanol in the reduced atmosphere. Flasks were kept in a shaking incubator at 100 rpm and 40°C for 2 h. The infusions were filtered with Whatman #1 until a clear extract was obtained. The extracts were evaporated to dryness in a multi evaporator (Syncore Polyvap, Mason Technology, Ireland) at 60°C at their respective pressure and stored at -20°C until used.

Determination of total polyphenolic content : Total polyphenolic content (TPC) of vegetable extracts was determined by the method of Jaiswal et al. (2012) using Folin-Ciocalteau's phenol reagent (Sigma-Aldrich, Germany). Results were expressed as mg gallic acid equivalents per gram (GAE/g) of extract (dw) through the calibration curve of gallic acid.

Determination of total flavonoid content: The total flavonoid content (TFC) was determined according to the method of Liu et al. (2009) and results were expressed as mg quercetin (Sigma-Aldrich, Germany) equivalents per gram (QE/g) of extract (dw).

DPPH free radical scavenging capacity: The DPPH radical scavenging effect of vegetable extracts was measured according to the method of Rajauria et al., 2010. The assay was performed in a 96-well round-bottom microplate (Sarstedt, Inc, USA) with 1:1 ratio of 100 µl each of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical solution (165 µM) and sample. The reaction mixtures were incubated for 30 min at 25°C in dark conditions, and absorbance was measured at 517 nm in a microplate reader (Powerwave, Biotek, VT, USA). The ability to scavenge the DPPH radical was calculated. Calculated EC_{50} values indicate the concentration of sample required to scavenge 50% DPPH radicals. Ascorbic acid was used as a reference compound.

Ferric reducing antioxidant potential (FRAP) assay: Total antioxidant (AO) power of samples were measured using FRAP assay according to the method reported by Benzie and Strain, 1996 with some modifications (Jaiswal et al., 2012). The working FRAP reagent was freshly prepared by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) (Sigma-Aldrich, Germany) in 40 mM hydrochloric acid and with 1 volume of 20 mM ferric chloride. Preheated 100 µl FRAP reagent at 37°C was dispensed in each well with 50 µl of samples or standard. The absorbance was read after 10 min at 593 nm with the help of microplate spectrophotometer. Trolox (Sigma-Aldrich, Germany) was used as a standard and the results were expressed as mg trolox equivalents per gram (mg TE/g) of extract (dw).

Statistical analysis: Results are expressed as mean values ± standard deviation (SD). Analysis of variance (ANOVA) and multiple comparisons (Fisher's least-significant-difference test) were used to evaluate the significant difference among various samples using STATGRAPHICS Centurion XV. Differences at $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

In the present study, two marine vegetables namely *Himanthalia elongata* and *Laminaria saccharina* were compared with terrestrial vegetables *Brassica oleracea* (brocolli and Brussels sprouts) based on its polyphenolic content and antioxidant capacity. Results showed that considerable variations in extraction yield among the samples (Table 1). The highest extraction yield was recorded in the marine vegetable *L. saccharina* followed by *H. elongata* whereas the lower yield was evident in terrestrial vegetables Brussels sprouts followed by Broccoli.

Table 1. Total phenol, flavonoid content and extraction yield of selected marine and terrestrial vegetables

Samples	Extraction yield (%)	TPC mg GAE/g (dw) extract	TFC mg QE/g (dw) extract
Terrestrial vegetables			
Broccoli	5.25 ± 0.22[a]	23.6 ± 1.75[a]	17.5 ± 1.25[a]
Brussels sprouts	6.89 ± 0.12[b]	20.4 ± 0.43[a]	15.4 ± 0.72[a]
Marine vegetables			
H. elongata	6.40 ± 0.39[b]	140.2 ± 2.43[b]	50.4 ± 2.17[b]
L. saccharina	8.90 ± 0.65[c]	36.3 ± 1.82[c]	25.2 ± 1.23[c]

TPC of the various vegetable extracts varied between 20.4 ± 0.43 to 140.2 ± 2.43 mg GAE/g of extracts (dw) (Table 1). *H. elongata* extract exhibited significantly ($p < 0.05$) higher TPC compared to *L. saccharina* while no significant ($p > 0.05$) difference was observed between broccoli and Brussels sprouts. Results showed the presence of considerable amounts of flavonoid in all the samples, which follows similar trend as TPC (Table 1). Among all the samples, marine vegetables had significantly ($p < 0.05$) higher TPC and TFC compared to both terrestrial vegetables, however considerable variation was observed in the TPC and TFC content of marine vegetables itself, which could be due to differences in habitats, changes in salinity, temperature, nutrients and UV-visible irradiation.

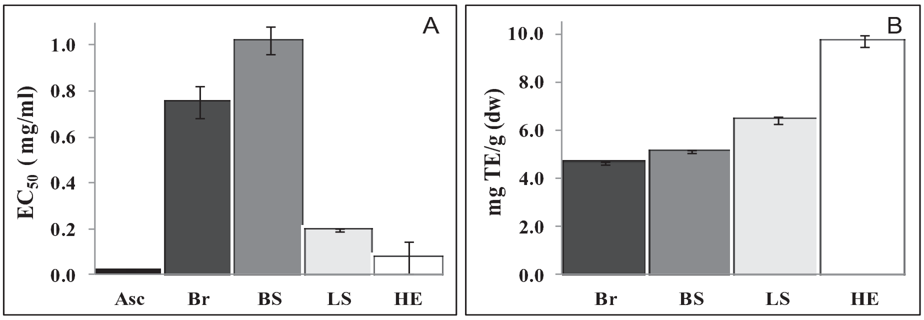

Figure 1. Antioxidant capacity of selected marine and terrestrial vegetables (1A) DPPH free radical scavenging capacity (expressed as EC_{50} values) of vegetables and ascorbic acid (reference compound) (1B) Total antioxidant capacity (trolox equivalent) of vegetable samples (Asc Ascorbic acid; Br Brocolli; BS Brussels sprouts; LS *L. saccharina*; HE *H. elongata*)

Two different methods [2,2-Diphenyl-1-picrylhydrazyl free radical scavenging capacity (DPPH RSC) and Ferric reducing AO potential (FRAP) assay were used for the estimation of total AO capacity of the samples. All the vegetable extracts, at the tested concentrations, were capable of directly reacting with and quenching DPPH radicals, but it was significantly ($p < 0.05$) lower than that of reference compounds ascorbic acid. Total antioxidant capacity of selected marine and terrestrial vegetables was estimated by FRAP assay. Total antioxidant capacity was observed in the range of 4.66 ± 0.07 to 9.70 ± 0.23. Among the samples, *H. elongata* showed the highest AO capacity which is confirmed by both the assay systems followed by *L. saccharina;* however, there was no significant difference observed in the total AO capacity of terrestrial vegetables. Brussels sprouts had higher DHPH radical scavenging capacity as compared to broccoli. Previous studies showed that phenol rings are important for the structural property of the polyphenolic compounds. It was reported that some of the marine vegetables have up to eight interconnected phenol rings (Hemat, 2007). They are therefore, more potent free radical scavenger than other polyphenols derived from terrestrial plants, which only have three to four rings (Hemat, 2007). The extensive mode of action of these phenolics is due to the substitutions on the aromatic ring which affect the stabilization and as a result the radical-quenching ability of these phenolic compounds.

CONCLUSIONS

Present study confirmed that both marine and terrestrial vegetables are rich in polyphenols and have appreciable amount of antioxidant property irrespective of their origin. However, it was seen that vegetables with marine origin has significantly higher polyphenols content and antioxidant property. These findings suggest that apart from the terrestrial vegetables, marine vegetables can also be considered as a potential source of natural antioxidants. Further investigations are underway for the identification and quantification of individual polyphenolic compounds and their various bioactive potential.

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