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Research Article

COMPARATIVE EFFICACY OF DIFFERENT *MATTEUCCIA STRUTHIOPTERIS* EXTRACTS AS AN ANTIOXIDANT IN RAW GROUND PORK DURING REFRIGERATED STORAGE

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ABSTRACT

The purpose of this study was to evaluate the oxidative and colour stability of raw ground pork incorporated with 1% (w/v) of different *Matteuccia struthiopteris* (Fiddle head fern) extracts viz. acetone (AF), ethanol (EF), methanol (MF) and water (WF) during refrigerated storage for 9 days. Total phenolic content, 1, 1 diphenyl-2-picrylhydrazil (DPPH) radicals and 2-2-azinobis-3-ethylbenthiazoline-6-sulphonic acid (ABTS+) radical activity were the highest in AF and the lowest in WF. Fiddle head (FH)-treated raw ground pork had significantly lower thiobarbituric acid reactive substances than control (raw pork samples without any treatment) during storage. Amongst treatments, the antioxidant efficacy followed an order of WF<MF<EF<AF. Raw ground pork meat incorporated with AF and EF exhibited a decrease in L^* Lovibond's colour values. The pH and a_w value were higher in control than FH-treated products and followed an increasing trend throughout the storage. Therefore, it can be concluded that acetone and ethanolic extracts of fiddle head fern can be used successfully as an antioxidant to minimize lipid peroxidation and colour deteriorative changes in raw ground pork products.

Key words: Fiddle head fern, antioxidants, *Matteuccia struthiopteris*, lipid oxidation

Lipid per oxidation is one of the major causes for deterioration and reduced shelf life of meat products (Fernandez-Lopez *et al.,* 2005). Ground meat tends to become brown and rancid more rapidly than whole muscle retail cuts (Ho *et al.,* 1996). Pork oxidizes more rapidly than beef and/or lamb. Concerns related to the safety and toxicity problems of synthetic antioxidants has led to increase in the interest for natural antioxidants. Moreover, an antioxidant added to a food to improve lipid stability may also help in reducing the oxidative stress on the consumers (Ramarathnam *et al.,* 1995).

A multitude of natural antioxidants have been isolated from different plant materials such as oilseeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs. Potent antioxidant capacities of all these plants are mainly due to their unique composition of flavonoids, carotenoids, vitamin C, vitamin E and phenolic compounds. Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness in food depends not only on the number and location of hydroxyl groups but also on factors such as physical location, interaction with other food components, and environmental conditions.

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Ferns and lichens have been used for food and medicines since ages (Nonato *et al.,* 2009). The immature fronds or fiddle heads of the ostrich fern (*Matteuccia struthiopteris* L; FH) have been consumed as a spring vegetable for generations (DeLong and Prange, 2008). The FH is recognized as a potential source for human nutrition and rich source of unique phenolic composition contributing to its antioxidants potential (DeLong *et al.,* 2011). The aim of this study was to investigate the antioxidant activity of different solvent extracts of FH and their effect on colour and oxidative stability of raw ground pork during refrigerated storage.

MATERIALS AND METHODS

Preparation of Different Fiddle Head Fern Extracts: Fiddleheads are harvested early in the season before the frond has opened to about 20 cm height. The curled crosiers (fiddleheads) of *M. struthiopteris* were collected from North-Western Himalayan region and were brought to the laboratory under refrigerated condition. The samples were manually cleaned, washed with water, cut into small pieces, dried overnight in an air drier at 55ºC, ground to a particle size of 25 mesh using a grinder, and

stored at 20ºC in an air tight container until use. Dried FH fern powder (10g) was extracted with 100 ml of 75% acetone, 75% ethanol, 75% methanol and hot water overnight in an orbital shaker at room temperature. The extracts were filtered through Whatman filter paper No. 42 and the solvents were distilled off in a rotary vacuum evaporator (RE-300 Yamato rotary evaporator, Japan) at 45°C on 100 rpm for 20 min. After evaporation of the respective solutes, the extracts were stored in amber coloured bottles at -20°C till further use. All extracts were analyzed for 2-2-azinobis-3-ethylbenthiazoline-6-sulphonic acid (ABTS+) radical cations (Re *et al.*,1999), 1, 1 diphenyl-2-picrylhydrazil (DPPH) radicals (Kato *et al.*, 1988) and total phenolic (Yuan *et al.*, 2005).

Preparation of Pork Meat Samples: Post-rigor deboned pork meat (mixture of *Musculus biceps femoris, M. semimembranosus, M. semitendinosus, M. gracilis and M. aductor*) were obtained from slaughter house of the department. Meat free from separable fat and connective tissue was minced through a 6 mm grinding plate in a meat mincer (MEW 714 MADO Eskimo, Germany). After mincing, meat samples were divided into five different batches viz. control (without any added extract), acetone extract of FH (AF), ethanol extract of FH (EF), methanol extract of FH (MF) and water extract of FH (WF) and 1% w/v of each extract was added in respective batches which were mixed thoroughly in a domestic food blender (Inalsa India Limited, Delhi) for 2 min. After mixing, each batch was aerobically packaged in low density polyethylene bags. The samples were stored at 4±1ºC and they were drawn at 2 days interval (1st, 3rd, 5th, 7th and 9th day) for evaluation of 2- thiobarbituric acid reacting substances (TBARS) values (Witte *et al.*, 1970), water activity (Rotonix HYGRO Palm AW1 Set/40), pH (Trout *et al.*, 1992) and Lovibond colour values (Lovibond RT-300, Reflactance Tintometer, UK).

Factorial design with six replicates was employed for storage data (pH, water activity, TBARS and colour values) with treatments and storage period using two-way analysis of variance. Analysis of variance was performed on all the variables using the General Linear Model procedure of the SPSS statistical package (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test ($P<0.05$) was used to determine level of significance between treatment means.

RESULTS AND DISCUSSION

The comparative efficacy of antioxidant activity of different FH extracts is presented in Fig. 1. The results revealed that DPPH and ABTS+ activities varied significantly with the type of solvent. The lowest activity was recorded in WF whereas the highest was in AF. The ABTS+ activity (Fig 1a) was significantly higher in AF (83.92%) as compared to EF (81.82%), MF (72.37%) and WF (62.19%). Similaraly, the DPPH scavenging activity (Fig 1b) was 62.19, 68.87, 74.24 and 79.32% in WF, MF, EF and AF, respectively.

The content of extractable phenolic compounds in extracts (µg GAE/g) were determined by a regression equation of the calibration curve (y=0.0016x–0.0317, R2=0.9962) and were 66.73 (AF), 56.14 (EF), 48.45

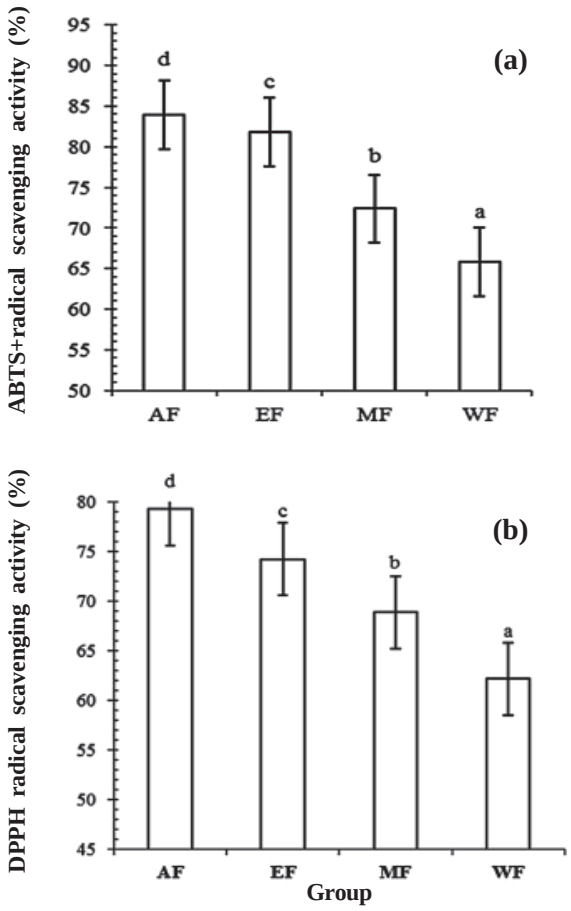

Fig 1. ABTS+radical (a) and DPPH radical (b) scavenging activity (%) of different extracts [AF= Acetone extract of FH; EF= Ethanolic extract of FH; MF= Methanolic extract of FH and WF = Water extract of FH. a–d Mean±SE with different small letter superscripts are different significantly ($P< 0.05$)].

(MF) and 45.52 (WF). Total phenolic content varied in the order of AF>EF>MF>WF. These results are in consonance with DPPH and ABTS+ activities. Higher value of AF might be due to more extraction of biologically active components from the plant matrix with acetone. Plant phenols and polyphenolic compounds are widely distributed in natural herb and fern extracts and they have been found to possess significant antioxidant activity (DeLong *et al.*, 2011).

Perusal of Table 1 revealed that pH of raw ground pork varied significantly amongst treatments and storage days. The pH was comparable in AF, EF and MF and

Table 1
Effect of various Fiddle head fern extracts on colour attributes, pH, TBARS and a_w values and colour attributes of raw pork meat during refrigeration storage (4±1ºC).

Parameter	Treatments	Storage period (days)				
		1	3	5	7	9
pH	Control	5.82±0.02^{bB}	5.83±0.01^{bAB}	5.81±0.01^{aC}	5.97±0.05^{cC}	6.12±0.12^{dD}
	AF	5.78±0.01^{bcA}	5.81±0.01^{bcA}	5.70±0.02^{aA}	5.76±0.01^{bA}	5.82±0.03^{cA}
	EF	5.79±0.02^{aA}	5.81±0.03^{bcA}	5.79±0.05^{bB}	5.83±0.01^{bcBC}	5.84±0.01^{cB}
	MF	5.80±0.03^{aA}	5.82±0.01^{bcA}	5.81±0.01^{bC}	5.84±0.02b^{cBC}	5.86±0.04^{cC}
	WF	5.81±0.04^{aAB}	5.84±0.01^{bB}	5.86±0.02^{bcD}	5.97±0.05^{cC}	6.06±0.02^{dD}
TBARS values	Control	0.675±0.0 1^{aC}	0.693±0.16^{aB}	0.981±0.01^{bC}	1.030±0.01^{cE}	1.326±0.01^{dD}
(mg MDA/kg)	AF	0.583±0.01^{aA}	0.597±0.02^{aA}	0.615±0.01^{aA}	0.782±0.01^{bA}	0.798±0.01^{cA}
	EF	0.590±0.01^{aAB}	0.598±0.02^{aA}	0.645±0.00^{bA}	0.838±0.01^{cB}	0.849±0.01^{dB}
	MF	0.598±0.01^{aAB}	0.601±0.00^{aA}	0.779±0.01^{bB}	0.912±0.01^{cC}	0.974±0.01^{dB}
	WF	0.613±0.00^{aB}	0.634±0.01^{aA}	0.782±0.01^{bB}	0.988±0.00^{cD}	1.111±0.02^{dC}
a_w	Control	0.936±0.03^{dA}	0.882±0.02^{cA}	0.862±0.03^{bcA}	0.823±0.03^{bA}	0.745±0.02^{aA}
	AF	0.984±0.05^{cD}	0.935±0.03^{bcD}	0.907±0.02^{bBC}	0.873±0.03^{aD}	0.882±0.01^{aD}
	EF	0.981±0.02^{dCD}	0.926±0.01^{cdD}	0.896±0.00^{bcC}	0.861±0.06^{bBC}	0.851±0.02^{aCD}
	MF	0.976±0.03^{dC}	0.911±0.01^{cC}	0.883±0.03^{bcB}	0.854±0.02^{bC}	0.842±0.01^{aC}
	WF	0.964±0.02^{dB}	0.887±0.02^{cdB}	0.878±0.02^{cAB}	0.841±0.02^{bB}	0.797±0.02^{aB}
L^* value	Control	51.41±1.12^{aB}	50.45±0.99^{aB}	51.28±0.60^{aB}	55.35±1.49^{bA}	59.07±0.74^{cB}
	AF	58.41±0.48^{abD}	57.61±0.43^{cD}	52.01±0.42^{bB}	59.41±0.08^{cBC}	48.19±0.55^{aA}
	EF	52.45±0.97^{bcB}	51.76±0.94^{bB}	54.49±0.49^{cC}	57.98±0.21^{dBC}	49.51±0.71^{aA}
	MF	55.02±0.25^{bC}	54.11±0.14^{bC}	52.62±0.57^{aB}	57.62±0.19^{cB}	58.58±0.74^{cB}
	WF	46.04±0.76^{abA}	45.16±0.72^{aA}	48.67±0.11^{cA}	59.90±0.16^{dC}	47.52±1.04^{bcA}
a^* value	Control	7.56±0.36^{bB}	7.20±0.33^{bB}	7.40±0.19^{bB}	7.39±0.49^{bAB}	5.99±0.09^{aA}
	AF	9.44±0.15^{cdC}	10.00±0.23^{dC}	5.30±0.15^{aA}	6.89±0.20^{bA}	8.77±0.51^{cB}
	EF	6.35±0.10^{aA}	6.74±0.03^{bAB}	6.04±0.03^{aB}	7.55±0.17^{cAB}	9.03±0.18^{dB}
	MF	6.30±0.06^{bA}	6.75±0.09^{cAB}	6.71±0.02^{cC}	7.99±0.11^{dB}	5.84±0.19^{aA}
	WF	5.95±0.17^{aA}	6.42±0.15^{aA}	6.72±0.15^{abC}	7.34±0.14^{bAB}	8.92±0.49^{cB}
b^* value	Control	12.46±1.61^{aA}	12.00±1.75^{aA}	14.45±0.24^{aB}	14.86±0.15^{aA}	18.16±0.10^{bB}
	AF	18.58±0.14^{dB}	18.43±0.23^{dB}	13.79±0.33^{aA}	17.40±0.11^{cB}	15.87±0.55^{bA}
	EF	14.12±0.43^{aA}	13.83±0.27^{aA}	15.18±0.04^{bC}	17.43±0.19^{dB}	16.40±0.54^{cA}
	MF	14.87±0.28^{aA}	14.59±0.23^{aA}	14.65±0.15^{aCD}	17.88±0.16^{bBC}	18.22±0.36^{bB}
	WF	13.20±0.24^{aA}	12.76±0.36^{aA}	15.46±0.07^{bD}	18.26±0.20^{dC}	16.98±0.70^{cAB}
Hue Value	Control	62.69±1.15^{aA}	67.86±0.94^{bB}	72.47±0.77^{cB}	77.76±0.74^{dD}	62.78±1.11^{aA}
	AF	71.77±0.54^{cB}	72.97±0.79^{cC}	77.34±0.61^{dC}	62.69±1.15^{aA}	67.86±0.94^{bC}
	EF	73.94±0.53^{cB}	75.99±0.43^{dD}	64.47±0.84^{aA}	71.73±0.54^{bB}	72.97±0.79^{bcE}
	MF	76.67±0.61^{cC}	65.39±0.65^{aA}	66.47±0.84^{aA}	73.94±0.53^{bC}	75.99±0.43^{cD}
	WF	62.78±1.11^{aA}	68.14±0.82^{cB}	73.60±0.38^{dB}	76.67±0.61^{eD}	65.39±0.65^{bB}
Chroma value	Control	12.94±0.13^{eE}	9.52±0.08^{cC}	8.23±0.16^{bB}	7.39±0.16^{aA}	10.55±0.21^{dD}
	AF	10.01±0.21^{dC}	8.33±0.10^{bB}	7.43±0.10^{aA}	12.94±0.13^{eE}	9.52±0.08^{cC}
	EF	9.16±0.05^{cB}	7.51±0.08^{aA}	9.87±0.07^{dC}	10.01±0.02^{dD}	8.33±0.10^{bB}
	MF	8.01±0.22^{bA}	10.50±0.12^{eD}	8.47±0.18^{cB}	9.16±0.05^{dC}	7.51±0.08^{aA}
	WF	10.55±0.19^{cD}	9.24±0.01^{bC}	8.26±0.22^{aB}	8.01±0.22^{aB}	10.50±0.12^{cD}

n=6; control=without any treatment; AF=Acetone extract of FH; EF=Ethanolic extract of FH; MF=Methanolic extract of FH and WF=Water extract of FH. $^{a–c}$ Mean±SE with different small letter superscripts in a row are significantly different (P<0.05). $^{A–D}$ Mean±SE with different capital letter superscripts in a column are significantly different (P<0.05).

was significantly ($p<0.05$) lower than WF and control at all days of observation during storage. During storage, the pH slightly decreased in treated products upto 5th day and increased thereafter. Variation in pH values may be attributed to pH of solvents used and bacterial metabolites produced during storage. It can also be correlated with the water activity (a_w) level in the raw ground pork in different treatments, which directs the microbial growth in the products. The a_w was found to be the lowest in control and the highest in AF followed by EF, MF and WF at day 9 of storage (Table 1). The a_w decreased significantly in all the treatments including control during storage.

TBARS values represent the content of secondary lipid oxidation products, mainly aldehydes (malonaldehyde), which contribute to off-flavours in oxidized meat and meat products. Statistical analysis of the data indicated that TBARS values of all FH-treated samples was significantly lower ($P<0.05$) than the control during storage (Table 1). Among the treatments, AF recorded the lowest, whereas WF recorded the highest TBARS values at 9th day of storage. It can be attributed to greater inhibitory effect of AF on lipid oxidation of raw ground pork during storage. These results can be correlated with the total phenolic constituents of Fiddle head fern (Table 1). In general, lipid oxidation increased during storage due to denatured structure of muscles under aerobic storage condition (Witte *et al.*, 1970). FH extracts were able to check the rise in TBARS values indicating antioxidant properties of FH.

The instrumental L^* value (lightness) decreased ($P<0.05$) with the addition of the AF, WF and EF (Table 1). Inclusion of MF resulted in a slight increase in L^* values. Redness (a^*) values of AF and MF showed a decreasing trend, whereas EF and WF showed an increasing trend during storage. Redness of the control and WF decreased while that of AF and EF increased significantly ($P<0.05$) during storage; it might be due to oxidation of the myglobin. Addition of AF and EF stabilized the redness value in comparison to control after 7 and 9 days of storage. Results can be correlated with antioxidant potential of the extracts. Yellowness (b^*) values of control and MF were higher ($P<0.05$) than AF and EF. In addition, AF has recorded the lowest b^* values at the end of storage (Table 1). Hue and Chroma values for WF and control showed non-significant differences during storage as compared to 1st and 9th day of storage. Hue values showed an increasing trend in WF and a decreasing trend in AF, EF and MF during storage. Chroma values were increased in all samples excluding WF.

The results demonstrate that Fiddle head fern extracts are a rich source of phenolic compounds with significant DPPH and ABTS+ scavenging activity. Among all extracts, AF and MF were effective in reducing malonaldehyde formation of raw ground pork during aerobic storage. Therefore, it is concluded that Fiddle head fern could be successfully incorporated in raw ground pork as a natural antioxidant to extend shelf life.

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