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Phytochemical and Antioxidant Activity Variation of Processed Edible Ferns

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ABSTRACT

The main and interaction effects on the total phenolic content (TPC), total flavonoid content (TFC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical seavenging activity of two edible ferns in various food processing methods were investigated. Factors include fern species (Diplazium eculentum and Stenochlaena palustris), and type of processing methods at various levels (blanching at 80^{0}C for 1, 2 and 3 minutes, boiling at 4, 5 and 6 minutes; freezing at 1, 2 and 3 weeks; microwave at 30, 60 and 90 seconds; oven dry at 70^{0}C for 1, 3 and 5 hours; steaming at 8, 9 and 10 minutes; vacuum packaging at 1, 2 and 3 weeks and fresh as control) were studied. Main effects were based on fern species, process and levels while, interaction effects comprise the combinations of the main effects. Results revealed that the main and interaction effects to all parameters used were significantly different. D. esculentum has a higher TPC and TFC than S. palustris. However, S. palustris attained higher DPPH radical scavenging activity than D. esculentum. Among the processing methods, oven drying attained high TPC and TFC while steaming increased the DPPH radical scavenging activity. Both of these processes were best at low levels.

Keywords: edible ferns, phenolics, flavonoids, antioxidant activity

INTRODUCTION

The Philippine archipelago is one of the most important biodiversity hotspots on earth with high amounts of endemic plant and animal species (Langenberger et al., 2006). In the Philippines, about 930 species of ferns with more than 60 species having definite or probable usages (Amoroso and Amoroso, 2003). Many species can be considered as sources of food, raw materials for handicraft manufacture, medicine, organic fertilizer, building materials, potting medium and as ornamentals (Amoroso and Amoroso, 2003). These species have been known for their antioxidant activities based on different studies of different researches. Antioxidant activity had been a help to fight against radical damages, which might result in degenerative human complications. One way to combat radical damages is to improve body antioxidant status, which could be accomplished by higher consumption of vegetables and fruits of high antioxidant content. Foods from plant origin usually contain natural antioxidants that can cleanse free radical (Amin et al., 2006). However, green leafy vegetables, such as edible ferns are not usually consumed directly, unlike fruits. Prior to its consumption, these vegetables usually undergo a heating process. Blanching, boiling, microwave and steaming are considered thermal processing and can greatly affect the plant material to cause changes in its physical state and chemical properties.

Food processing methods involve several processes such as freezing, the use of pressure, plasma and many others. These processes are usually applied to ensure the safety of the food products. Food processing methods, when applied to leafy vegetable, renders it to be more edible and less toxic but may have a drastic decrease in the antioxidant property (Oboh, 2005). While plenty of evidence flourishes on the antioxidant capacity of tropical fruits, there is lack of evidence on antioxidant and phytoconstituents existing in tropical green leafy vegetables with their phytochemicals and antioxidant capacity when the processing operation is employed. Hence, the study of two edible ferns, D. esculentum and S. palustris were conducted to evaluate the effects of the different

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methods and levels of food processing operations on the TPC, TFC and DPPH radical scavenging activity.

METHODOLOGY

Materials, Chemicals, and Equipment

Two species of ferns were used in this study namely: D. esculentum and S. palustris. The equipment used were thermometer, steamer, microwave, oven, blender, pipettor and analytical balance. The Folin-Ciocalteu reagent, quercitin, gallic acid, ascorbic acid, DPPH, aluminum chloride, sodium acetate, sodium carbonate, methanol, and ethanol used were purchased from Sigma- Aldrich®. Other materials such as glass jars, Erlenmeyer flask, test tubes, test tube racks, volumetric flask, sterilized pipette tips, 96-wells microplate, and spatula were needed. The absorbance was read using a Molecular devices® Spectra Max 250 microplate reader.

Plant Materials

The ferns were freshly picked early in the morning at the University Fernery located in the campus of Central Mindanao University, University Town, Musuan, Maramag, Bukidnon, Philippines and at Mt. Musuan fernery.

Sample Extraction

Fern samples were processed according to the types of processing methods involved with its respective levels (blanching 80°C at 1, 2 and 3 minutes; boiling at 4, 5 and 6 minutes; freezing at 1, 2 and 3 weeks; microwave at 30, 60 and 90 seconds; oven dry at 70°C for 1, 3 and 5 hours; steaming at 8, 9 and 10 minutes; vacuum packaging at 1, 2 and 3 weeks and fresh as control). Processed fern samples were extracted using analytical grade absolute methanol following the method of Nayak et al. (2009). The methanolic extract was prepared by homogenizing 30 grams of the fern sample with 150 mL of methanol and allowed to stand for 48 hours. The methanolic extract was filtered using Whatman no. 42 filter paper. The filtrate was then subjected to phytochemical analysis.

Phytochemical Screening Method

Determination of Total Phenolic Content (TPC)

A modified method according to Chatatikun and Chiabchalard (2013) was used for the TPC determination using the Folin- Ciocalteu method. Methanolic plant extract of 200 µL (2.5 mg/mL) was transferred to a microcentrifuge tube. Similarly, gallic acid (300 ppm) as the standard solution with different concentrations (0, 10, 20, 25, 30, 35, 40, 50 µg/mL) was transferred into a microcentrifuge tube. The extracts and standard solutions were added separately with 200 µL of 10% Folin- Ciocalteu reagent followed with 800 µL of 700 mM sodium carbonate solution. The microcentrifuge tubes were incubated at room temperature for 2 hours. After incubation, the microcentrifuge tubes were centrifuged at 11,000 rpm for 2 minutes. Then, 200 µL of the supernatant was transferred to the assigned microplate wells. The absorbance of the reaction mixture was measured at 750 nm using UV Spectrophotometer microplate reader. The total phenolic content for the methanolic plant extract was determined and was expressed as mg Gallic Acid Equivalent (GAE) per kilogram of plant sample.

Determination of Total Flavonoid Content (TFC)

The modified method according to Chatikun and Chiabcharlard (2013) was used for the TFC determination using the aluminum chloride colorimetric assay. Methanolic plant extracts of 50 µL (2.5 mg/mL) were transferred into the assigned microplate wells. Quercetin (100 ppm) dissolved in 80% methanol was used as the standard solution with different concentrations of 6.25, 12.5, 25, 50, 100 µg/mL. The standard solutions were transferred into the assigned microplate wells. 10 µL of 10% aluminum chloride solution, followed by 130 µL of 95% methanol and 10 µL of 1 M sodium acetate was added to the mixture in the microplate wells. All reagents were mixed and incubated at room temperature for 40 minutes in the dark. The absorbance of the reaction mixture was measured at 415 nm using the UV Spectrophotometer microplate reader. The total flavonoid content of the methanolic plant extract was determined and was expressed as mg Quercetin Equivalent (QE) per kilogram of plant sample.

DPPH Radical Scavenging Activity Determination

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the extract was determined using the method of Mosquera et al. (2007) as described by Amoroso et al. (2014). Briefly, 1mL of methanolic sample extract was diluted with 1mL methanol. In A 96-well template, 75 µL of the resulting solution was transferred to the assigned wells and was further diluted with 100 µL methanol. It was added with 75 µL DPPH solution (20mg/L methanol). Ascorbic acid (400ppm) and methanol served as positive and negative control, respectively. Blanks for each samples were prepared by adding 75 µL extract and 175 µL methanol. The mixture was incubated at room temperature for 30 min in the dark. The plate was shaken for 10 seconds in the microplate reader before measuring the absorbance

at 517 nm. The scavenging activity was calculated using Eq. 1:

% DPPH radical scavenging activity=(Absorbance DPPH-(Absorbance DPPH+sample/Absorbance sample))/(Absorbance DPPH-Absorbance Ascorbic acid)×100

Experimental Design and Statistical Analysis

Fern species (D. esculentum and S. palustris), type of processing with respective levels (blanching 80°C at 1, 2 and 3 minutes; boiling at 4, 5 and 6 minutes; freezing at 1, 2 and 3 weeks; microwave at 30, 60 and 90 seconds; oven drying at 70°C for 1, 3 and 5 hours; steaming at 8, 9 and 10 minutes; vacuum packaging at 1, 2 and 3 weeks and fresh as control) were used as experimental factors. Data were analyzed using Statistica 6.0 through a Balanced Analysis of Variance (ANOVA) and Tukey's Honestly Significant Difference Test for the comparison between treatments to determine the main and interaction effects of the variables employed affecting the TPC, TFC and DPPH radical scavenging activity of the fern species used

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RESULTS AND DISCUSSION

Main Effects

It is known that phenolic compounds as a source of antioxidants are ubiquitous in plants that are an essential part of the human diet, and are of considerable interest due to their antioxidant properties (Balasundram et al., 2006). According to Heim et al. (2002), the antioxidant activity poses a greater contribution to the beneficial effects derived from phenolic compounds. Comparison between fern species has been reported by Amoroso et al. (2014) that D. esculentum has higher phenolic content than S. palustris. The same findings were also reported by Semwal et al. (2013) that D. esculentum showed the strongest overall antioxidant compared to other pteridophytes. These results are in agreement to the present study wherein D. esculentum showed a higher amount on the TPC, TFC, and DPPH scavenging activity (expressed as ascorbic acid equivalent) regardless of the process and levels employed in the experiment (Table 1). The lower values obtained from S. palustris suggest that the fern species has likely undergone oxidation when subjected to various processing operations and the levels applied.

Methods on the processing of ferns revealed that oven dried samples were consistently highest in TPC and TFC except for the DPPH scavenging activity as shown in Table 1. According to Capecka et al. (2005), the drying process may result to high or low levels of TPC which depends on the type of

Table 1. *Main effects on the total phenolic content, total flavonoid content and DPPH radical scavenging activity*

Effects	Total Phenolic content (mg GAE/g)	Total flavonoid content (µg QE/g)	DPPH radical scavenging activity expressed as Ascorbic acid equivalent (mg AEAC/g)
Fern species			
D. esculentum	5.99±0.58[a]	1.55±0.11[a]	1.45±0.13[a]
S. palustris	5.34±0.68[b]	0.93±0.11[b]	1.33±0.08[b]
Type of food processing			
Blaching	4.16±0.39[g]	1.16±0.38[d]	1.59±0.05[c]
Boiling	4.69±0.38f	1.08±0.28e	1.63±0.05b
Freezing	3.67±1.44h	1.09±0.17e	1.19±0.05g
Microwave	5.22±0.7d	1.21±0.30cd	1.46±0.11d
Oven dried	8.49±1.51a	1.81±0.26a	1.20±0.20f
Steaming	5.12±1.71e	1.23±0.25	1.68±0.04a
Vacuum pack	6.18±1.06	1.28±0.21b	0.93±0.36
Fresh	7.74±0.32b	1.06±0.17e	1.39±0.23E
Levels			
Low	6.37±0.87a	1.29±0.19a	1.40±0,08b
Medium	5.39±0.73b	1.16±0.16b	1.35±0.
High	5.23±0.56c	1.16±0.16b	1.43±0.11a

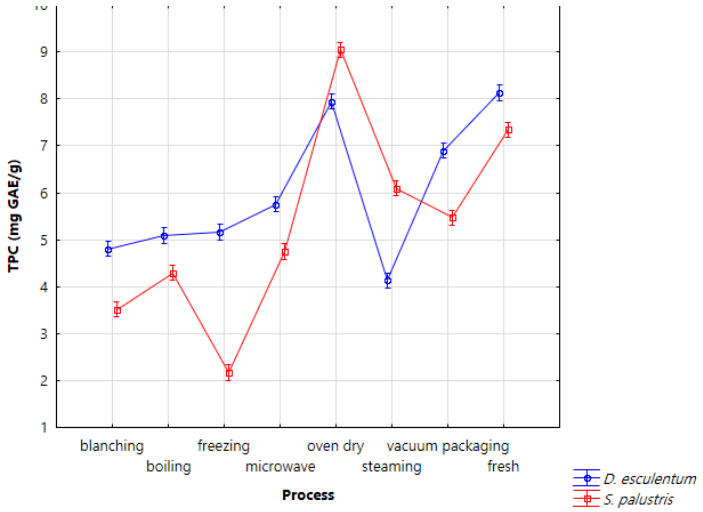
Means with the same letter are not significantly different between treatments in the column at $p<0.05$.

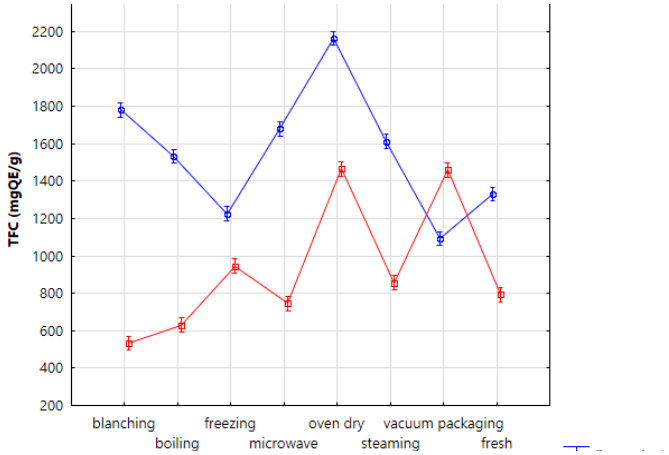
phenolic compounds present in the plant material and their location in the cell. For example, related studies revealed an increase in polyphenolic content after drying which has been reported for tomatoes (Chang et al., 2006) and shiitake mushroom (Choi et al., 2006). TPC in vegetables can be affected by different drying methods as reported by Hung and Duy (2012). Specifically, free and bound forms of vegetables' TPC were determined using freeze and heat drying methods. Their findings implied that the phenolic compounds in vegetables existed primarily in free form rather than in bound form. In addition, free phenolics were significantly higher in freeze-dried vegetables, their bound phenolics were slightly lower as compared with the heat-dried vegetables. Bound phenolics were not affected by drying methods because of their association with the cell wall of vegetables.

Results also revealed that at low levels of processing, there was a significant increase in TPC and TFC values. This is in contrast to the DPPH radical scavenging activity, which favors the high levels employed in this study. Treatments at low temperature and short time might have a minimal loss of antioxidant properties as compared to higher temperature and longer time. This is speculated to be due to minimal thermal degradation and leaching into the water, resulting in solubilization of phenolic compounds (Jaiswal et al., 2011). Moreover, a significant increase in the DPPH radical scavenging activity at a high level was observed. This is due to the increased solubilization of ferulic acid sugars with both increased heating time and temperature, thus, enhancing the antioxidant activity of the commodity. Thermally processed commodity might retain or increase its total phenolics and total antioxidant activity despite the loss of vitamin C (Dewanto et al., 2002). Correspondingly, the bound phenolic content decreased as they were released from esterified and insoluble-bound forms. Thermal processing may release more bound phenolic acids from the breakdown of cellular constituents, although disruption of cell walls would also release oxidative and hydrolytic enzymes that can destroy the antioxidants in fruits and vegetables (Chism and Haard, 1996; Dewanto et al., 2002). In addition, thermal processing at a higher temperature will deactivate these enzymes, thus preventing the loss of phenolic acids.

Interaction Effects

Interaction effects with fern species and





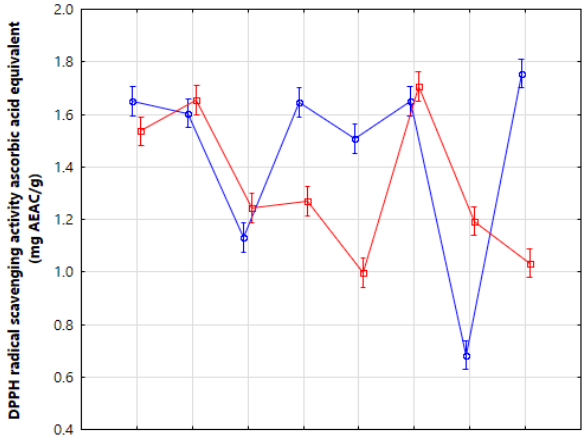


Figure 1. Interaction effects of fern species and the processing methods involved affecting the a. TPC b. TFC and c. DPPH radical scavenging activity

the processing methods as factors exhibited great significance to TPC, TFC, and DPPH scavenging activity as shown in Figure 1. Results revealed that there was a decrease in the total phenolic content of the D. esculentum when subjected to various processing, as compared with the fresh sample (control). A decrease in TPC values were noted in steaming (3.99 mg GAE/g sample) followed by blanching (3.32 mg GAE/g sample), boiling (3.04 mg GAE/g sample), freezing (2.97 mg GAE/g sample), microwaving (2.38 mg GAE/g sample), vacuum packaging (1.23 mg GAE/g sample) and oven-drying (0.19 mg GAE/g sample). In contrast, S. palustris exhibited a decrease of TPC with the following processing methods: freezing (5.18 mg GAE/g sample), blanching (4.19 mg GAE/g sample), boiling (3.06 mg GAE/g sample), microwaving (2.58 mg GAE/g sample), vacuum packaging (1.88 mg GAE/g sample) and steaming (1.88 mg GAE/g sample) except in oven-drying with an increase of 1.69 mg GAE/g sample.

D. esculentum showed gains and losses in TFC values with the types of processing involved. Among all types of processing operations, vacuum packaging (0.24 mg QE/g sample) and freezing (0.11 mg QE/g sample), showed a decrease in TFC compared to the fresh sample. However, an increase in TFC was observed with oven drying (0.83 mg QE/g sample), blanching (0.45 mg QE/g sample), microwaving (0.35 mg QE/g sample), steaming (0.28 mg QE/g sample) and boiling (0.20 mg QE/g sample). On the other hand, S. palustris showed a decrease on TFC with blanching (0.26 mg QE/g sample), boiling (1.16 mg QE/g) and microwaving (0.04 mg QE/g sample) but there was an increase in TFC in oven drying (0.68 mg QE/g sample), vacuum packaging (0.67 mg QE/g sample), freezing (0.16 mg QE/g sample) and steaming (0.07 mg QE/g sample) compared to the fresh sample.

The interaction of fern species and types of processing resulted to a higher TPC on S. palustris and TFC for D. esculentum both on oven dried. This increase would be associated with various reasons. According to Rhandhir et al. (2007), phenolic compounds are usually present in bound states as conjugates with sugars, fatty acids or proteins. It could be speculated that the disassociation of these complexes followed by some polymerization of the phenolic contents may be responsible for the increase on the antioxidant capacity. The increase on flavonoid content could be due to the absence of the enzymatic oxidation, caused by enzymes, which prevent the loss of the antioxidant compounds in the heat- treated plant metabolites (Dewanto et al., 2002). Microwave treatment for a short time would inactivate enzyme that would cause degradation of antioxidant properties (Ravichandran et al., 2013). The increase of antioxidant activity on microwave treatment was in agreement with the results from Dewanto et al. (2002a); Dewanto et al. (2002b). Furthermore, thermal processing increases the total antioxidant activity, which could be due to their different vitamin contents, and phenolic compounds, which can act synergistically but are then sensitive to processing.

The interaction effects between fern species and different levels of temperature also showed a significant effect on the samples as shown in Figure 2 except on TPC. Results revealed that D. esculentum showed a higher value of both TPC and TFC at a lower level while S. palustris had a higher value of antioxidant activity at low levels similar to that of D. esculentum.

Temperature is an important factor for antioxidant stability (Ravichandran et al., 2013). Results showed that at low levels, high antioxidant properties was observed in both fern species. The results suggest that the loss in water-soluble antioxidant was minimal. On the contrary, prolong thermal treatment resulted to decrease in antioxidant activity in both plant species suggesting that there was a loss of antioxidant enzyme activity that could also be accompanied by loss of bioactive compounds.

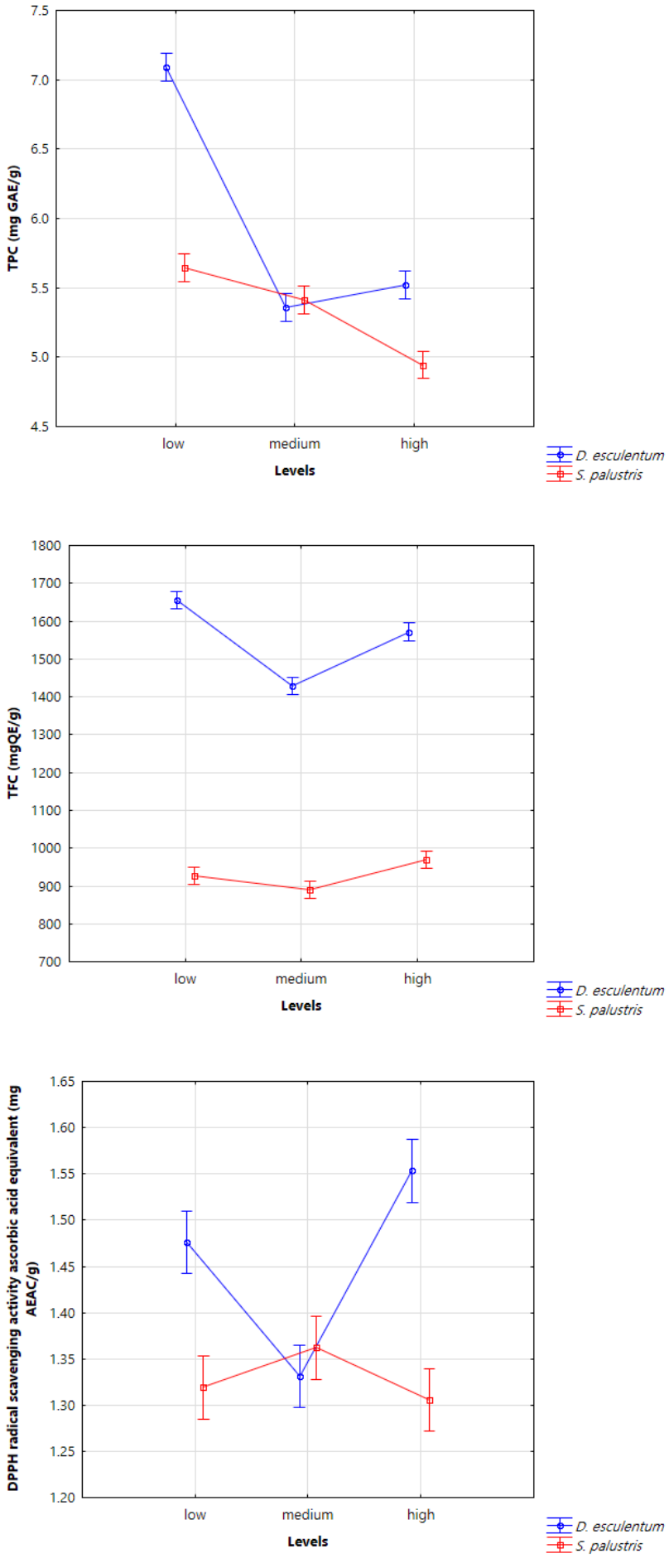
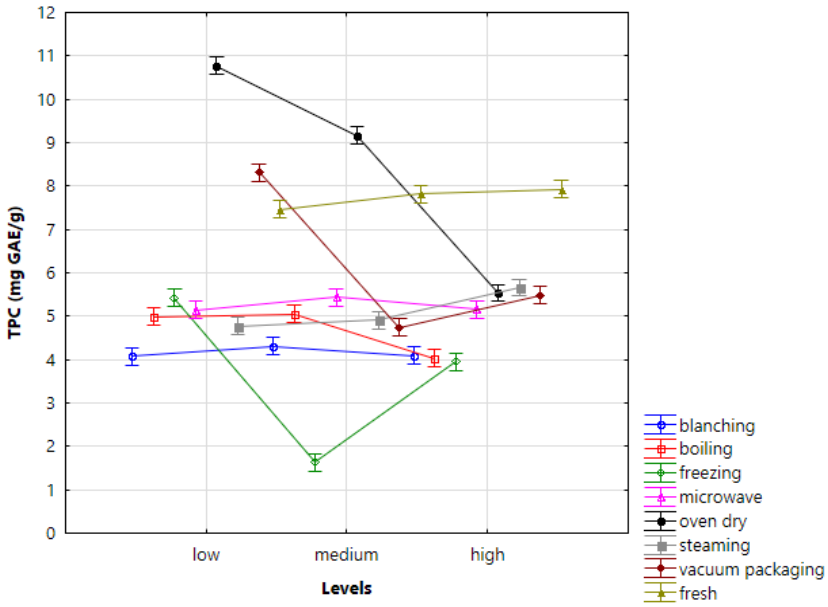


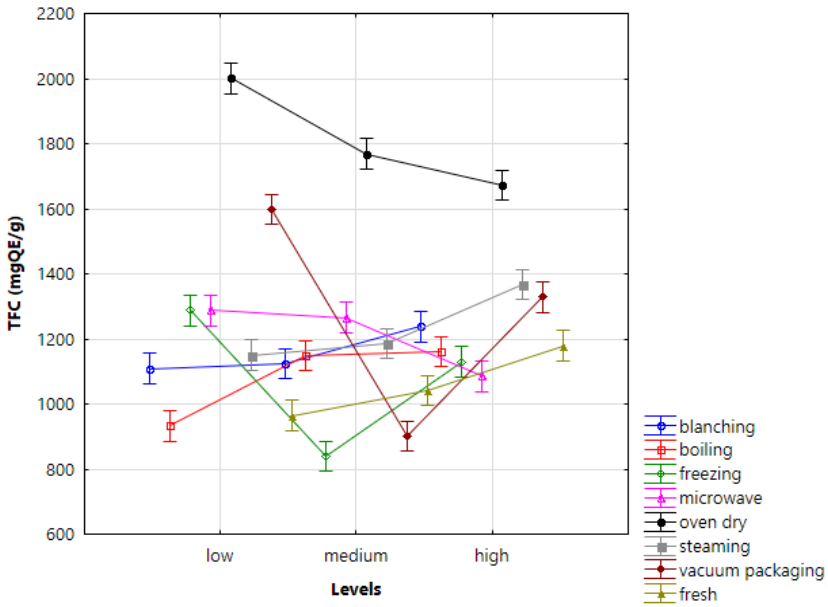
Figure 2. Interaction effects of fern species and levels involved affecting the a. TPC b. TFC and c. DPPH radical scavenging activity

The interaction between the type of processing methods and levels showed that among the processes and levels, there was an increase in both TPC and TFC as observed in the oven-dry treatment at a low level (Figure 3). Similarly, an increase in antioxidant activity was observed on microwave treatment at a high level. This is mainly due to the collapse of the intercellular spaces of the plant which will eventually liberate phenolic compounds (Hoissain et al., 2010).

Phenolic compounds are antioxidants and are subject to oxidation during storage and processing of foods (Titchenal and Dobbs, 2004). The major contribution to the antioxidant activities of plant foods is related to their content of polyphenols. Therefore, it is important to consider the effect of each treatment applied in the process upon retaining the antioxidant properties of the plant material (Jaiswal et al., 2011). Phenolic compounds are usually susceptible to different factors during the extraction process. For example, using a low temperature for drying yields the highest amount of phenolic content. However, increasing the temperature decreased the values of the phenolic content of the sample. At higher temperature, certain phenolics may decompose or react with other plant components (Jaiswal et al., 2011).

The interaction effect between processing methods and different levels of temperature also gives a significant effect on the plant sample. Results showed that among processing operations, oven drying at low level showed higher values to both TPC and TFC. While steaming at medium level revealed a higher value of DPPH radical scavenging activity. The





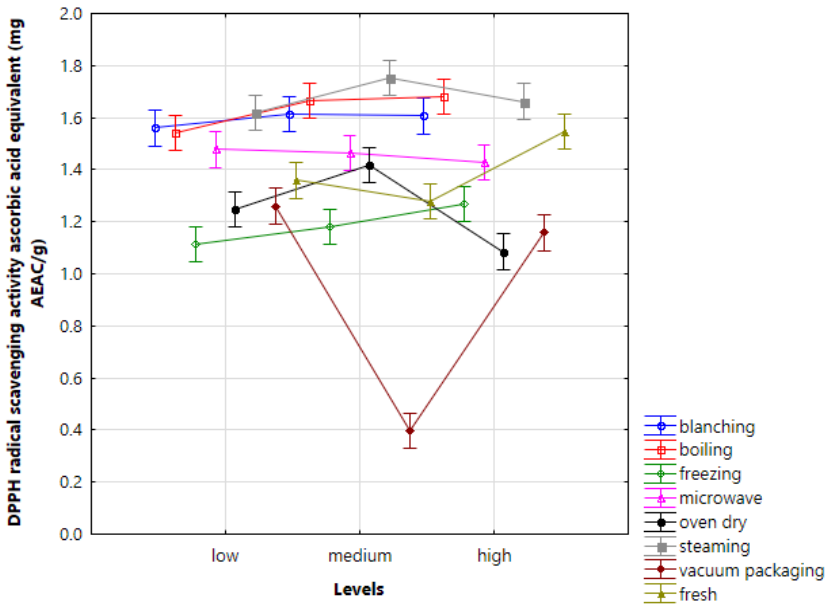
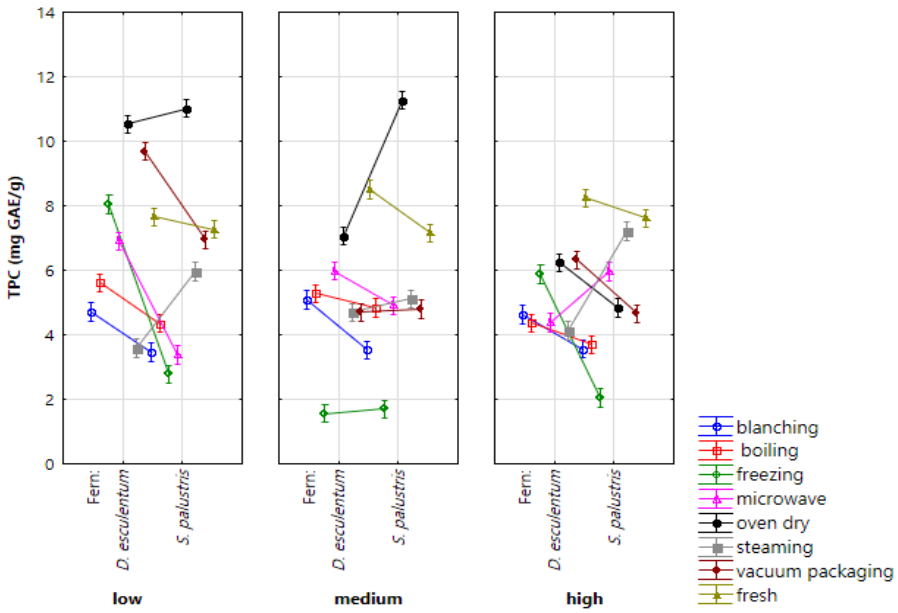


Figure 3. Interaction effects on the types of processing methods and levels involved affecting the a. TPC b. TFC and c. DPPH radical scavenging activity

increase of antioxidant activity at medium level is in agreement to the study of Ravichandran et al. (2013), wherein the longer time of processing yields an increase in the antioxidant activity. The increase in antioxidant activities of treated samples shows that the antioxidant activity depends on the other polyphenols, which could have been increased during the design of treatments.

Figure 4 shows the interaction between the three factors that include: fern species, type of processing methods and the levels. These three factors greatly affect the antioxidant properties of the fern samples. The noticeable effect of gains and losses of antioxidants was observed. Results revealed that S. palustris exhibited highest TPC when subjected to oven drying at a medium level of temperature D. esculentum on the other hand, showed the highest TFC content at high levels of oven drying while high values for DPPH radical scavenging activity when subjected to steaming at a medium level. In this case, D. esculentum is more prone to thermal degradation than that of the S. palustris. Since phenolic compounds are unstable at high temperature, thus could be easily degraded. This increase of TPC is mainly due to the drying at a medium temperature because at favorable temperature results a high yield of phenolics (Miean and Mohamed, 2001).



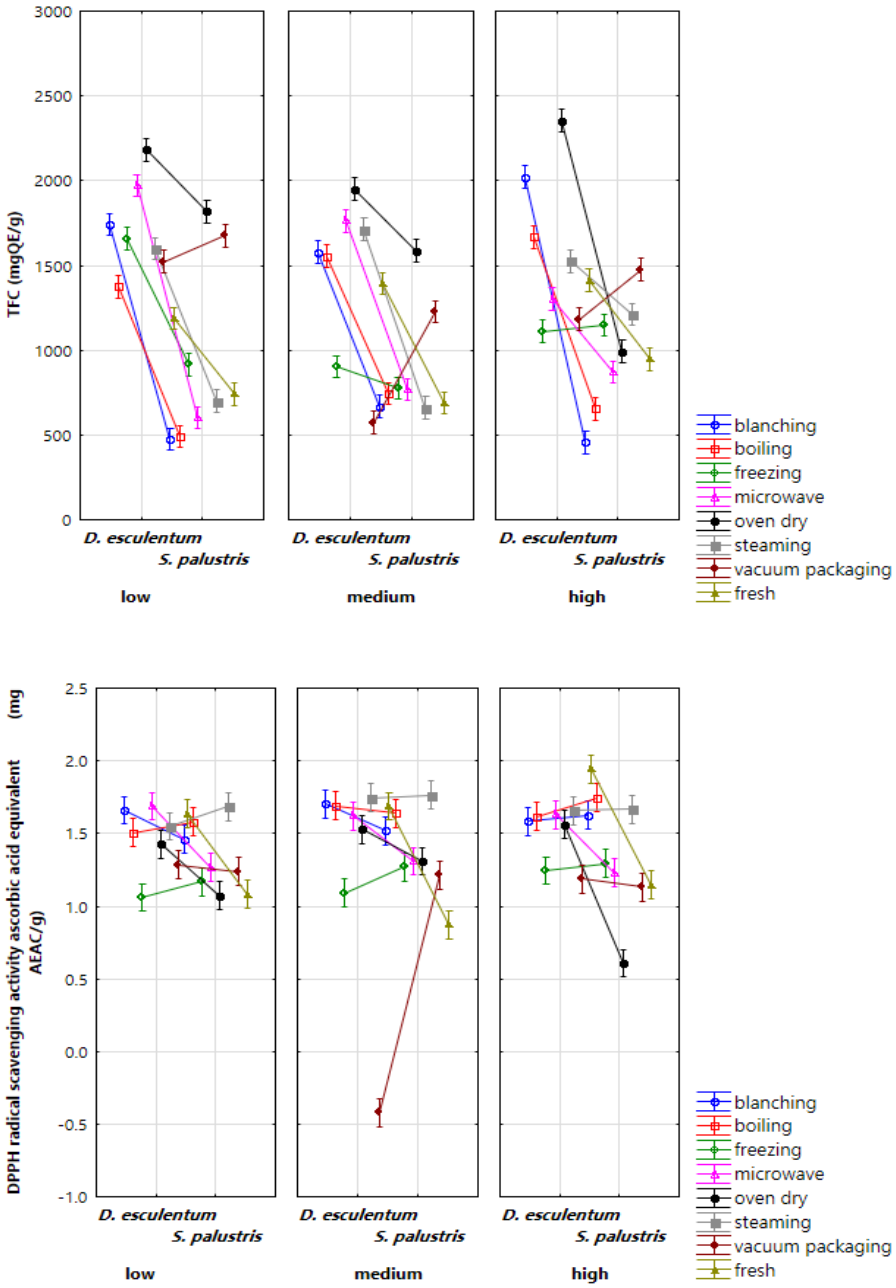


Figure 4. Interaction effects on the fern species, types of processing methods and levels involved affecting the a. TPC b. TFC and c. DPPH radical scavenging activity

Results of the study also revealed that S. palustris has high antioxidant activity on microwave treatment at a high level. The result suggests that at a high temperature of microwave treatment, there was an increase in the antioxidant activity and produced many useful metabolites that include antioxidants. These useful metabolites are considered to be a high specific metal chelating activity (Chai et al., 2012), which are likely not prone to immediate degradation.

CONCLUSIONS

The main effects revealed that D. esculentum showed a higher antioxidant property compared to S. palustris. TFC values were affected to processing methods used with an increase during oven-drying Similarly, DPPH radical scavenging activity showed the highest activity in steaming.

Interaction effects based on species and process revealed that among the combined processes, an increase of TPC was observed to S. palustris when subjected to oven drying while considering an increase in TFC was also accounted for D. esculentum when subjected to oven drying DPPH radical scavenging activity showed that D. esculentum increased in most

processing operations, except for freezing at low and medium levels, and for vacuum packaging at high level. For S. palustris, a decrease in radical scavenging activity was observed when oven dried at a high level but exhibited a significant increase in most processing operations.

RECOMMENDATIONS

The researchers would like to recommend oven drying and steaming as processing methods on ferns, but other factors or combinations of food applications such as pressure as a factor should be taken into account. It is also recommended that prolong storage of sample and processing under higher temperature are not advisable.

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