Assessment of the antioxidant and pro-oxidant activities of tree nut extracts with a pork model system

R. Amarowicz[1] & R.B. Pegg[2]

[1]Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Tuwima 10, 10-747 Olsztyn, Poland; E-mail: amaro@pan.olsztyn.pl.

[2]Department of Food Science and Technology, The University of Georgia, 100 Cedar Street, Athens, GA, 30602-7610, USA; E-mail: rpegg@uga.edu.

Abstract

Crude extracts of phenolic compounds were prepared using 80% (v/v) acetone from the residues of almonds, hazelnuts, pecans, and walnuts after oil extraction. A fraction comprising low-molecular-weight phenolic compounds (I) and a tannin fraction (II) were obtained from each crude extract *via* Sephadex LH-20 column chromatography. The antioxidant activities of these preparations were evaluated using a pork model system. When tested at 100, 200, and 500-ppm levels, almond and hazelnut crude extracts exhibited a slight, but noticeable pro-oxidant activity as assessed by an increase in TBARS, whereas walnut and pecan crude extracts exhibited a dose-dependent antioxidant efficacy, and were superior to a commercial preparate of natural antioxidants. The tannin fraction isolated from walnut and pecan offered strong antioxidant properties at all levels tested, with a concentration as low as 100 ppm providing complete protection to the meat lipids. Chromatography suggests that hydrolyzable tannins present in these nuts rather than proanthocyanidins are likely responsible for the observed efficacy in the cooked pork systems. Thus, phenolic compounds recovered from specialty oil processing of walnut and pecan may be used as a natural additive in the development of functional meat products to impart antioxidant activity and radical scavenging capacity.

Background

During production, processing, distribution and storage, meat undergoes deterioration from chemical and microbial processes. Typically, oxidative deterioration of meat results from degradative reactions of lipids in raw and thermally-processed products. The rate and extent of oxidative deterioration can be reduced by various means such as curing to preserve the meat tissue, vacuum packaging to remove the oxygen source, or addition of primary antioxidants to scavenge the oxidants. Over the past few years, antioxidants from natural sources have received an avalanche of print and media coverage on account of their alleged nutritional and health benefits. Polyphenolic compounds present in plant material are believed to play an important role in the antioxidant story. It is well documented that extracts of green tea, and spices such as rosemary, sage, oregano, thyme, and clove possess marked antioxidative activity. Research has led to the identification of (bio)active compounds in green tea and rosemary: these extracts have been successfully commercialized in Japan, Europe, and North America, despite higher costs in comparison to widely used synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ). Because BHA and BHT are suspected to possess carcinogenic activity, this has resulted in an increased effort by researchers to identify antioxidants from natural sources and to explore their potential food applications.

The objective of this study was to evaluate the polyphenolics extracted from the residues of selected tree nut species after oil extraction as potential sources of natural antioxidants in meat and meat products.

Materials & methods

Acetonic extracts (80% v/v) were prepared from the residues of almonds, hazelnuts, pecans, and walnuts after oil extraction. Each crude tree nut extract was fractionated by Sephadex LH-20 column (30 x 700 mm) chromatography using 95% ethanol [*i.e.*, low-molecular-weight {LMW} phenolic compounds, fraction (I)] and then 50% acetone [*i.e.*, tannins, fraction (II)] (Strumeyer and Malin, 1975). Total phenolics content was determined by the classical Folin-Ciocalteu assay, and condensed tannins according to Price *et al*. (1978).

Fresh pork cushions were ground twice by passing them through a 1/8O plate using an industrial meat grinder. Meat was transferred to jars, mixed with 20% (w/w) deionized water and various additives. The systems were thermal processed in an 85^{o}C water bath with occasional stirring by a glass rod until an internal temperature of 72∀1EC was reached. Systems were cooled to room temperature, homogenized in a blender for 30 s, transferred to Whirl Pak bags and refrigerated at 4EC until analyzed. 2-Thiobarbituric reactive substances (TBARS) were determined by the trichloroacetic acid method as described by Pegg (2005).

Results & discussion

The total phenolics content (TPC) of each crude tree nut extract and its LMW & tannin fractions are reported in Table 1. The TPCs of the crude pecan and walnut extracts (125 & 109 mg/g extract, respectively) were significantly greater than those of the almond and hazelnut extracts (16 & 65 mg/g extract, respectively). Yet, this trend did not follow for the content of condensed tannins in the nut extracts: the crude hazelnut extract, for example, contained the greatest amount of condensed tannins, but this did not translate to strong antioxidant activity, as determined in the pork model systems (see discussion below). The condensed tannins content in the tannin fractions of almond and pecan were similar, and those of hazelnut and walnut were much higher and of equal value. Low TPC and condensed tannins values were noted in all LMW fractions.

Table 1. Content of total phenolics and condensed tannins in nut extracts

Material Analyzed	Total phenolics (mg (+)-catechin/g extract or fraction)	Condensed tannins (Absorbance at 500 nm/g)
Almonds		
Crude extract	16	28
LMW fraction	7	2
Tannin fraction	80	296
Hazelnuts		
Crude extract	65	111
LMW fraction	16	–
Tannin fraction	330	961
Pecans		
Crude extract	125	76
LMW fraction	61	1
Tannin fraction	471	370
Walnuts		
Crude extract	109	35
LMW fraction	48	–
Tannin fraction	546	961

TBARS values of cooked pork treated with a commercial natural antioxidant (*i.e.*, oregano) and a second system without any treatment were compared against samples to which the tree nut extracts/fractions had been added at various concentrations. When tested at 100, 200, and 500-ppm levels, the crude extracts prepared from almond and hazelnut residues exhibited a slight, but noticeable pro-oxidant activity as indicated by an increase in TBARS values vs. the no additive control. On the other hand, crude extracts from walnut and pecan residues exhibited a dose-dependent antioxidant efficacy at the concentrations studied. The TBARS values for these systems were much lower than those of the no additive control, thereby indicating protection to the meat lipids against autoxidation from constituents in the extracts.

Figure 1 depicts the change in TBARS values of pork systems treated with 500 ppm of each crude tree nut extract and 1000 ppm of the natural antioxidant preparate over 14 days of 4°C storage. The 500-ppm data were chosen to illustrate the oxidation trends; lower addition levels of the crude walnut and pecan extracts (*i.e.*, 100 and 200 ppm) resulted in weaker antioxidant activity than 500-ppm treatments, as one would expect for a dose-dependent effect. The almond and hazelnut extracts imparted a pro-oxidative effect toward the meat lipids even at lower addition levels. This was surprising as antioxidant activity of almond and hazelnut extracts have been reported by Amarowicz *et al*. (2005) and Alasalvar *et al*. (2006): these extracts showed antiradical activity against $DPPH^{\bullet}$, $ABTS^{\bullet+}$, reducing power, and antioxidant properties in an emulsion system. Lack of radical scavenging or hydrogen atom transferring efficacy from the phenolic constituents in these nut extracts suggests a different class of phenolic constituents which (1) are non-effective in meat or (2) lose the antioxidative potential of active constituents during thermal processing.

An antioxidative efficacy was noticeable at 100- and 200-ppm levels of the LMW fraction from walnut and pecan, but at a 500-ppm addition level meat lipids were protected against oxidation during storage at 4°C to a similar extent as that of 100-ppm TBHQ and better than the commercial preparate employed at a 1000-ppm level (results not shown). The tannin fraction isolated from walnut and pecan offered strong antioxidant properties at all levels tested, with a concentration as low as 50 ppm providing complete protection to the meat lipids during 14 days of 4°C storage. Chromatography suggests that hydrolyzable tannins present in these nuts, rather than proanthocyanidins (*i.e.*, condensed tannins), are likely

responsible for the observed efficacy in the cooked pork systems. This may explain why the hazelnut extract, which was richest in proanthocyanidins, did not bestow potent antioxidant activity.

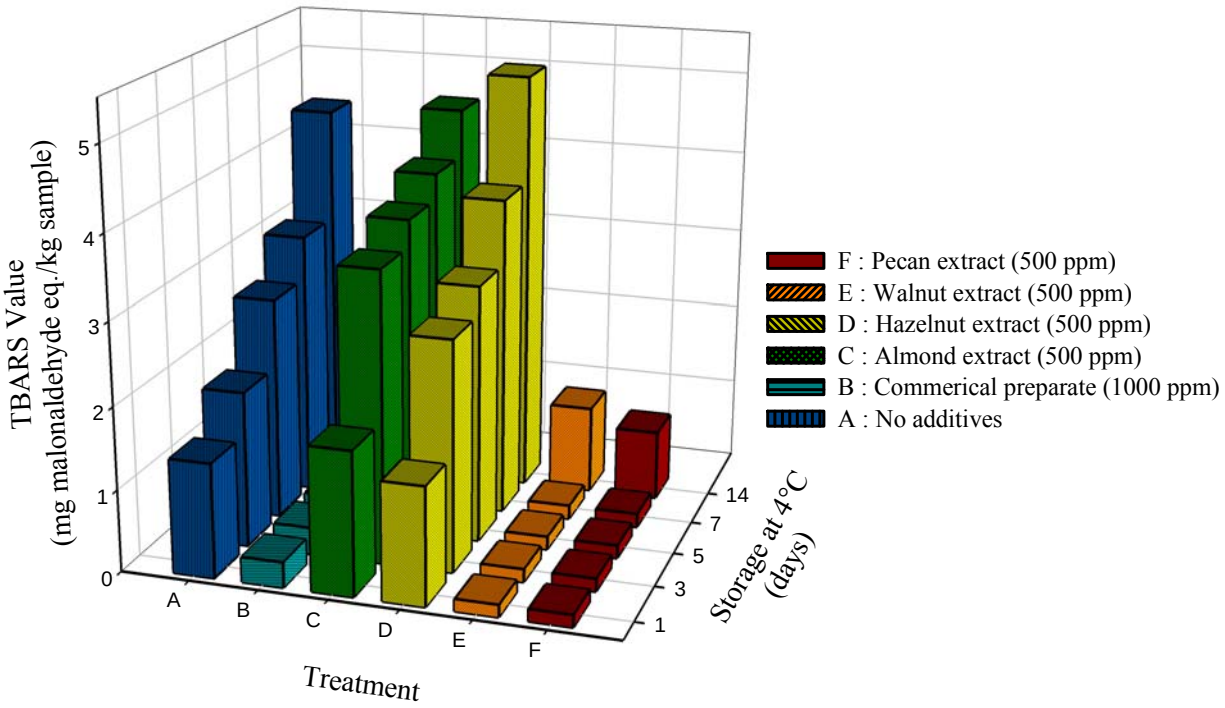

Figure 1. TBARS values of cooked pork systems as affected by selected nut extracts.

Conclusions

While all of the tree nut extracts and fractions were expected to demonstrate an antioxidant potential to varying degrees in the pork model systems, efficacy from almond and hazelnut extracts was lost when applied to meat systems. This was not the case for the walnut and pecan extracts and their fractions, which showed excellent thermal stability in cooked meat systems. Therefore, phenolic compounds recovered from specialty oil processing of walnut and pecan may be used as a natural additive in the development of functional meat products to impart antioxidant activity and radical scavenging capacity.

Acknowledgements

The authors wish to thank the assistance of Kimberly A. Lockin, a student in UGA's College of Agricultural and Environmental Sciences "Young Scholars' Internship Program," for her technical assistance.

References

Alasalvar, C., Karamać, M., Amarowicz, R., Shahidi, F., Antioxidant and antiradical activities in extracts of hazelnut kernel (Corylus avellana L.) and hazelnut green leafy cover. J. Agric. Food Chem., 2006, 54, 4826-4832.

Amarowicz, R., Troszyńska, A., Shahidi, F., Antioxidant activity of almond seed extract and its fractions. J. Food Lipids, 2005, 12, 344-358.

Pegg, R.B. 2005. Spectrophotometric measurement of secondary lipid oxidation products. In: Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M.H., Reid, D.S., Schwartz, S.J., Shoemaker, C.F., Smith, D.M. and Sporns, P. (eds.), Handbook of Food Analytical Chemistry. Water, Proteins, Enzymes, Lipids, and Carbohydrates, 547-564. New York, NY: John Wiley & Sons, Inc.

Price, M.L., Van Scoyoc, S., Butler, L.G., A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem., 1978, 26, 1214-1218.

Strumeyer, D.H., Malin, M.J., Condensed tannins in grain sorghum: Isolation, fractionation, and characterization. J. Agric. Food Chem., 1975, 23, 909-914.