



Roast effects on the hydrophilic and lipophilic antioxidant capacities of peanut flours, blanched peanut seed and peanut skins

J.P. Davis^{a,b,*}, L.L. Dean^{a,b}, K.M. Price^a, T.H. Sanders^{a,b}

^a USDA-ARS, Market Quality and Handling Research Unit, Raleigh, NC, USA

^b Department of Food, Bioprocessing and Nutrition Services, North Carolina State University, Raleigh, NC 27695-7624, USA

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ABSTRACT

Hydrophilic and lipophilic oxygen radical antioxidant capacity (H&L-ORAC) of peanut flours, blanched peanut seed, and peanut skins were characterised across a range of roast intensities. H-ORAC ranged from 5910 to 7990, 3040 to 3700 and 152,290 to 209,710 $\mu\text{moles Trolox}/100\text{ g}$ for the flours, seed, and skins, respectively. H-ORAC increased linearly with darker seed colour after roasting at 166 °C from 0 to 77 min, whereas skin H-ORAC peaked after roasting for 7 min. Linear correlations with H-ORAC and total phenolic content were observed. Additionally, completely defatted peanut seed were solubilised (5% w/w) in water and H-ORAC measured. For these samples, H-ORAC decreased with roast intensity which correlated with soluble protein. L-ORAC ranged from 620 to 1120, 150 to 730 and 2150 to 6320 $\mu\text{moles Trolox}/100\text{ g}$ for peanut flours, seed, and skins, respectively. L-ORAC increased linearly with both darker seed colour and skin colour across the 77 min range. L-ORACs of roasted peanuts and ingredients are discussed in terms of tocopherol contents and Maillard reaction products.

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1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are naturally present throughout the human body. The concentration of these reactive species can be exacerbated due to a number of factors, including exposure to environmental contaminants, radiation, and the onset of certain disease states, to name a few (Blomhoff, Carlsen, Andersen, & Jacobs, 2006). ROS/RNS can react nonenzymatically with a host of cellular molecules including lipids, proteins, nucleic acids, and carbohydrates, with these reactions being collectively termed 'oxidative stress'. Oxidative stress has been directly associated with the occurrence of multiple diseases, including various cancers, arthritis, and some forms of cardiovascular disease, to name a few (Blomhoff, 2005; Blomhoff et al., 2006). Elaborate systems within the human body have evolved to minimise oxidative stress and in addition to these natural systems, dietary intake is thought critical to maintaining a proper oxidative balance within the various tissues. Accordingly, research into dietary intake as related to the prevention of oxida-

tive stress within the human body is extensive with a number of foods having been studied in detail (Manna et al., 1997; Wu et al., 2004a). In addition to being important from a nutritional perspective, the antioxidant capacity of an ingredient is also important from a food stability perspective, as oxidation of foods often leads to unacceptable flavours, colours, or loss of nutrients (O'Keefe & Wang, 2006; StAngelo, 1996). As such, accurate measurements of antioxidant capacity are critical for both nutritional and food science applications.

Numerous assays for measuring antioxidant capacity have been reported, each with advantages and disadvantages; however, over the past few years the oxygen radical adsorption capacity (ORAC) assay has been established as a preferred antioxidant assay within the food industry. Important advantages of the ORAC assay include that it accounts for both inhibition time and inhibition degree within a single measurement, and the assay is appropriate for measurements of both hydrophilic and lipophilic antioxidant components (Prior, Wu, & Schaich, 2005; Wu et al., 2004b, 2004c). Additionally, an online USDA database has been established with ORAC data for approximately 277 foods and ingredients (USDA, 2007).

The positive health benefits associated with the regular consumption of tree nuts and peanuts (actually legumes) are well

* Corresponding author. Address: USDA-ARS, Market Quality and Handling Research Unit, Raleigh, NC, USA. Tel.: +919 515 6312.

E-mail address: jack.davis@ars.usda.gov (J.P. Davis).

established (Blomhoff et al., 2006; Isanga & Zhang, 2007; Talcott, Duncan, Del Pozo-Insfran, & Gorbet, 2005a). Peanuts, in addition to having healthy fatty acid profiles and being good sources of fibre and protein, contain a number of components that are capable of directly scavenging free radicals. Examples include various polyphenolics (coumaric acid, ferulic acid, resveratrol, etc.), tocopherols, flavonoids (procyanidins, catechin, etc.) and folate (Blomhoff et al., 2006; Isanga & Zhang, 2007). Less studied from a nutritional aspect are peanut flours and peanut skins. Peanut flours are commercially available high protein, low fat ingredients prepared from roasted peanut seed. Depending on the application, a variety of roast intensities and residual oil contents are available. Recent research has focused on characterising the functional properties of these ingredients including rheological, foaming, emulsifying, water holding capacity, etc. (Davis, Gharst, & Sanders, 2007; Ferreyra, Kuskoski, Luiz, Arellano, & Fett, 2007; Gharst, Clare, Davis, & Sanders, 2007); however, the antioxidant capacities of peanut flours have not been reported. Peanut skin (testae or seed coat) is the protective layer immediately surrounding the peanut seed. Skins are consumed with some peanut based products including in-shell peanuts and some specialty types of peanut butters; however, the vast majority of peanut skins are removed (blanched) during processing. It is estimated that world production of peanut skins is on the magnitude of 750,000 tons annually, with the only current market being low value animal feed applications (Sobolev & Cole, 2004). Skins are established to be a rich source of phenolic compounds, including various procyanidins among others, which suggests there is excellent potential to produce nutraceutical ingredients from peanut skin (Nepote, Grosso, & Guzman, 2005; O'Keefe & Wang, 2006; Yu, Ahmedna, & Goktepe, 2005; Yu, Ahmedna, Goktepe, & Dai, 2006).

Roasting whole peanuts at 175 °C for 10 min to a Hunter *L*-value of approximately 50 was found to increase hydrophilic ORAC (H-ORAC) approximately 22% (Talcott, Passeretti, Duncan, & Gorbet, 2005b). In a separate study, the antioxidant capacity (determined by an end point free radical scavenging method) of skins from peanut seed blanched at 175 °C for 5 min was shown to be minimally affected (Yu et al., 2006). However, peanuts are roasted to a variety of intensities depending on the intended final product and application. Furthermore, we are unaware of any studies directly measuring the lipophilic ORAC (L-ORAC) of peanuts and peanut based ingredients as a function of roast intensity. As such, H-ORAC and L-ORAC were characterised for a range of peanut flours, blanched peanut seed and peanut skins roasted to different intensities.

Additionally, roasted seed were completely defatted and H-ORAC was determined for the water soluble fractions.

2. Materials and methods

2.1. Materials

Peanut flours, obtained from Golden Peanut Company (Alpharetta, GA), were classified by the manufacturer according to (1) roast level, either light or dark and (2) fat content, either 12% or 28% of the dry weight. The following abbreviations are used to designate these flours: light roast – 12% fat = LR12, dark roast – 12% fat = DR12, light roast – 28% fat = LR28 and Dark Roast-28% = Fat DR28. Medium-grade size, runner-type peanuts (*Arachis hypogaea* L., variety Georgia green) were obtained from a single harvested lot from the USDA, ARS, National Peanut Research Laboratory (Dawson, Georgia). The peanuts were harvested, cured, shelled, sized, and stored according to normal practices prior to delivery to Raleigh, N.C. All solvents used for extractions were reagent grade or higher purity and obtained from Fisher Scientific.

2.2. Peanut roasting

Peanuts were roasted in a lab-scale roaster (Despatch, Minneapolis, MN) at 166 °C from 0 to 77 min. Upon removal from the oven, roasted peanuts were cooled using forced ambient air, and skins were manually removed using gloved hands, collected and stored in sealed commercial bags. Colours of the blanched, roasted peanuts, as well as the separated skins, were determined using a Hunter Lab DP-9000 colorimeter (Hunter Associates Laboratory, Reston, Va., USA).

2.3. Extraction of flours and roasted peanuts

Samples were extracted using a Dionex (Sunnyvale, CA) ASE[®] 200 Accelerated Solvent Extractor (Wu et al., 2004c). Flours were used in commercial form, whereas roasted, blanched peanuts and skins were lightly ground to increase surface area of extraction. Current extraction procedures were previously utilised in studies that surveyed ORAC values for a broad range of foods and ingredients (Wu et al., 2004b, 2004c). Approximately 1.0 g of peanut flours or peanut seed, or 0.5 g of skins were analytically weighed and mixed with 25 g of sea sand. Samples and sand were transferred to a 22 mL extraction cell and were initially extracted with hexane:dichloromethane (1:1, Hex/Dc) followed by acetone:water:acetic acid (70:29.5:0.5, AWA). Extraction parameters were: pressure, 1500 psi; temperature, 70 °C (Hex/Dc extraction) and 80 °C (AWA extraction); 3 × 5 min cycles; flushing volume, 60%; and nitrogen purge time, 60 s. Hex/Dc extracts were dried under nitrogen and brought up in 10 mL of acetone prior to lipophilic-ORAC analysis. AWA extracts were brought to 50 mL total volume with AWA prior to hydrophilic-ORAC analysis.

2.4. Hydrophilic-oxygen radical absorbance capacity (H-ORAC) assay

The H-ORAC procedure was adapted from Prior et al. (2003). Previously reported assay conditions were used as a starting point, and the final conditions reported here were empirically chosen such that the linearity of the standard curve and sample responses were appropriate. Assays were prepared in Costar polystyrene flat-bottom black 96 microwell plates (Corning; Acton, Massachusetts). A sodium salt solution of Fluorescein (Reidel-deHaen; Seelze, Germany) was prepared daily at a final concentration of 70 nM in 75 mM phosphate buffer. Trolox (Aldrich; Milwaukee, WI) standards were prepared from 50 to 3.12 μM in phosphate buffer. 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH) (Wako; Richmond, VA) was prepared daily at a final concentration of 153 mM in phosphate buffer immediately prior to usage. Fluorescence was measured using the SAFIRE² microplate reader equipped with Magellan (v. 6.1) reader software (Tecan USA; Raleigh NC). Excitation and emission filter wavelengths were set at 483 ± 8 and 525 ± 12 nm, respectively.

The reaction was carried out in 75 mM phosphate buffer at pH 7.4 with a final reaction volume of 250 μL. Hydrophilic extracts were diluted using phosphate buffer (minimum 50X) to fall within the linear region of the Trolox standard curve. Diluted sample extracts and standards, both at 130 μL, were added to the wells followed by 60 μL of the Fluorescein solution, which was rapidly added via a multi-channel pipette. The plate containing only the samples and standards + Fluorescein was incubated in the SAFIRE² for 15 min at 37 °C. 60 μL of the AAPH solution was then rapidly added via a multi-channel pipette. Prior to the first measurement, samples were shaken for 5 s using a medium orbital intensity. Data were acquired over 90, 1 min kinetic cycles with a 5 s medium intensity orbital shaking between cycles. Data was reported as relative fluorescent units (RFU) ranging from 0 to 60,000 RFU and

exported into Microsoft Excel (Microsoft; Roselle, IL) for further analysis.

2.5. Lipophilic-oxygen radical absorbance capacity (L-ORAC) assay

The L-ORAC procedure was adapted from Prior et al. (2003). Solutions of 7% pharmaceutical grade randomly methylated beta cyclodextrin (Trappsol®; CTD, Inc.; High Springs, FL, USA) were prepared in 50% acetone:water (7% RMCD). Trolox standards were prepared from 200 to 1.56 μM in 7% RMCD. Lipophilic extracts were diluted using 7% RMCD (flours and blanched peanuts ~ 1:25, skins ~ 1:1000) to fall within the linear region of the Trolox standard curve. Fluorescein was prepared daily at a final concentration of 21.5 nM in 75 mM phosphate buffer. AAPH was prepared daily at a final concentration of 77 mM in phosphate buffer immediately prior to usage. Diluted sample extracts and standards, both at 25 μL , were added to the wells followed by 120 μL of the Fluorescein solution, which was rapidly added via a multi-channel pipette, followed by incubation in the SAFIRE² for 15 min at 37 °C. 80 μL of the AAPH solution was then rapidly added via a multi-channel pipette. Prior to the first measurement, samples were shaken for 5 s using a medium orbital intensity. Data points were acquired over 180, 1 min kinetic cycles with a 5 s medium intensity orbital shaking between cycles. Data handling and export were the same as reported for H-ORAC.

2.6. Preparation of water soluble defatted extracts

Whole peanut seed roasted to differing intensities and blanched were completely defatted using a Soxhlet apparatus and hexane in excess as the extraction solvent. Defatted samples were air dried after extraction and stored in sealed tubes at refrigeration temperatures until used. 5% w/w dispersions were prepared with defatted samples and deionized water. Dispersions were rapidly stirred for 1.5 h using a bench top stirrer followed by centrifugation at 5000g/15 min to remove insoluble material. pH of the water soluble fractions was measured and protein content determined via the bicinchoninic acid (BCA) Assay.

2.7. Total phenolic content

Total phenolic content of various extracts was determined colorimetrically using Folin–Ciocalteu reagent as described previously (Al-Farsi, Alasalvar, Morris, Baron, & Shahidi, 2005). Briefly, 20–500 μL of extract was mixed with 1.5 mL of Folin–Ciocalteu reagent that had been previously diluted 1:10 in deionized water. This mixture was then incubated for 5 min at 22 °C after which 1.5 mL of sodium bicarbonate (60 g/L) was added. This mixture was then incubated for 90 min at 22 °C and absorbance at 725 nm measured. Standard phenolic compounds ferulic acid, gallic acid and catechin were dissolved in equivalent solvent mixtures and used for calibration curves.

2.8. Tocopherol analyses

Tocopherols were analysed by HPLC using a Luna 5 μ silica column, 250 mm length, 4.60 mm i.d. (Phenomenex, Torrance, CA, Cat No. 00G-4274-EO) with a mobile phase of 1% isopropanol in hexane at a flow rate of 1.2 mL/min (Hashim, Koehler, Eitenmiller, & Kvien, 1993). Peanut oils were mechanically pressed from the roasted seed and analytically diluted directly into the 1% isopropanol/hexane solvent. Additionally, lipophilic extracts (1.2 mL) were dried under nitrogen at 40 °C and reconstituted 1:1 volumetrically with hexane containing 1% isopropanol prior to injection. Injection volume was 20 μL and the detector was a Waters 2487 Dual Wavelength Absorbance Detector set to 294 nm. Tocopherols were iden-

tified by comparison with standards purchased from Sigma (Sigma Chemical Corporation, St. Louis, MO). Standards of α , β , δ and γ tocopherol were diluted with hexane. Concentrations were determined using Beer's Law and the UV maxima of the solutions. Extinction coefficients were taken from the Merck Index. Concentrations of unknowns were calculated by comparison of peak areas and the calculated concentrations of the standard solutions. Standard curves of each isomer covered five orders of magnitude and bracketed all sample concentrations.

2.9. Sample handling and statistical analyses

All flours and roasted peanut samples were independently extracted a minimum of three times prior to ORAC analyses. Within a given ORAC experiment, samples were measured in triplicate. Statistical Analysis Software (v. 9.1) by SAS Institute Inc. (Cary, NC) was used for data analysis. Means were differentiated using PROC GLM and the Tukey multiple adjustment ($P < 0.05$).

3. Results and discussion

3.1. ORAC of peanut flours

H-ORAC of the flours, raw-blanched peanut seed and roasted-blanched peanut seed are presented in Fig. 1A. Peanut seed in Fig. 1A and B were roasted at 166 °C for 21.5 min to an *L*-value of 47.5 ± 1.0 , a roast intensity that is considered “typical” for many applications. Data is presented as μmole Trolox equivalents (TE) per 100 g (fresh weight), which are the standard units for the USDA database containing ORAC data for 277 different foods and ingredients (USDA, 2007). Trolox is a water soluble Vitamin E analog and is a standard antioxidant used for this assay. H-ORACs of all flours were significantly ($P < 0.05$) greater than the raw-blanched and roasted-blanched peanut samples. H-ORAC for the raw-blanched peanut sample was $3040 \pm 70 \mu\text{mole TE}/100 \text{ g}$, which is similar to the previously reported value of 2890 ± 140 for raw peanut seed (Wu et al., 2004b). H-ORAC for the roasted-blanched peanut sample was $3460 \pm 180 \text{ TE}/100 \text{ g}$, which is similar to the previously reported value of 3410 ± 190 for peanut seed (non high oleic) roasted to a similar intensity (Talcott et al., 2005b). H-ORAC for the flours ranged from 5910 ± 830 to $7990 \pm 670 \mu\text{M TE}/100 \text{ g}$ for LR28 and DR12, respectively. H-ORAC for the peanut flours ranged from 1.7 (LR28) to 2.3 (DR12) times greater than H-ORAC for the roasted peanut seed, and this increase is at least partially attributable to the decreased fat content of the flours as compared to the roasted seed (~50%), as H-ORAC should logically increase with decreasing fat content. Accordingly, when flours were compared at equivalent roast intensities, 12% fat flours had higher H-ORAC as compared to 28% fat flours for both light and dark roast flours (Fig. 1A). A roast effect was also observed for the flours, that is darker roast flours had higher H-ORAC scores than the lighter roast flours when compared at an equivalent fat content (Fig. 1). H-ORAC for roasted-blanched seed was not significantly ($P < 0.05$) different than that of raw-blanched seed despite an approximate 14% increase. This is primarily due to the larger means and variations observed for flours which were included in the statistical analyses. As discussed later, others have also previously observed an increase in H-ORAC of whole peanut seed after a typical roast treatment (Talcott et al., 2005a). Moisture content of the flours ranged from 2.1% to 2.2% for 28% fat flours and 1.5% to 1.9% for the 12% fat flours. Moisture content of the roasted, blanched seed was $1.2 \pm 0.1\%$, whereas that of the raw-blanched seed was $7.2 \pm 0.1\%$, meaning differences in H-ORAC where partially a function of moisture content, although these differences are not thought substantial when comparing only roasted samples (flour or seed). ORAC data is presented on a fresh

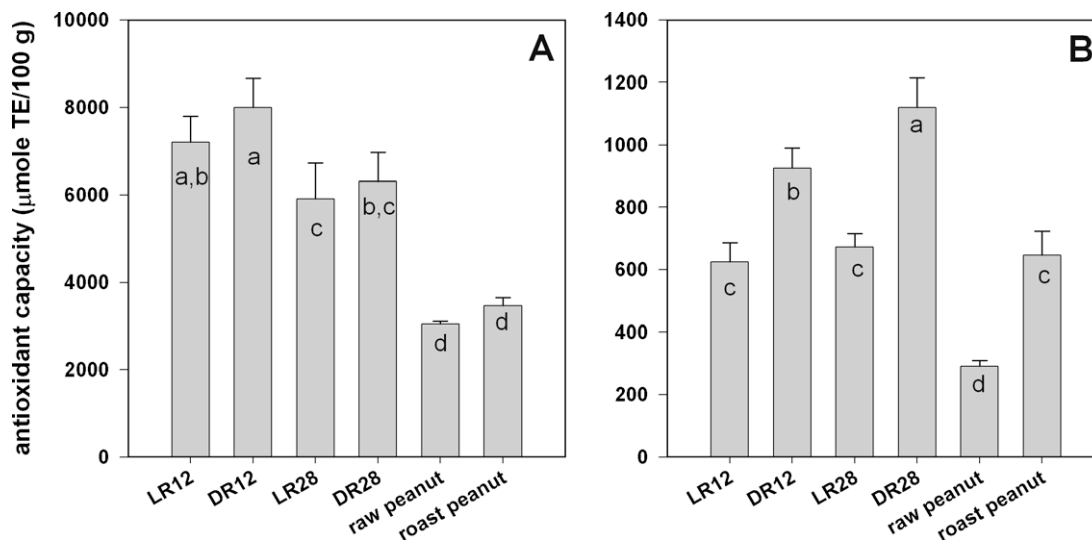


Fig. 1. H-ORAC (A) and L-ORAC (B) of peanut flours and raw-blanched peanut seed and roasted-blanched peanut seed. Light roast – 12% fat = LR12, dark roast – 12% fat = DR12, light roast – 28% fat = LR28 and dark roast – 28% = fat DR28.

weight basis for all samples, as these are the expected moisture contents for these products upon consumption and/or use as an ingredient.

L-ORAC for peanut flours, raw-blanched seed and roasted-blanched peanut seed are presented in Fig. 1B. L-ORAC for all flours were significantly ($P < 0.05$) greater than that of the raw-blanched peanut seed, despite the higher oil content of the peanut seed (~50%) as compared to the flours ($\leq \sim 28\%$). L-ORAC for the raw-blanched seed was 290 ± 18 $\mu\text{mole TE}/100$ g, which compares well to the value of 270 ± 60 $\mu\text{mole TE}/100$ g reported previously for raw seed (Wu et al., 2004b). Roasting significantly ($P < 0.05$) increased L-ORAC for the roasted-blanched peanut sample such that this value (650 ± 80 $\mu\text{mole TE}/100$ g) was equivalent to that of either LR12 or LR28. Values for the flours ranged from 620 ± 60 to 1120 ± 100 $\mu\text{mole TE}/100$ g for LR12 and DR 28, respectively. When compared at equivalent fat contents, dark roast flours had significantly ($P < 0.05$) greater L-ORAC than light roast flours, and the roasted-blanched peanut seed had a significantly ($P < 0.05$) greater L-ORAC than the raw-blanched peanut seed (Fig. 1). However, a fat effect was not necessarily apparent in the data, as the two light roast flours were not significantly ($P < 0.05$) different when compared across fat content, while L-ORAC of the 28% fat dark roast flour was significantly ($P < 0.05$) greater than the 12% fat dark roast flour (Fig. 1). Comparison with the USDA ORAC database found that all peanut flours had L-ORAC scores greater than roughly 85% of the 164 foods and ingredients listed (USDA, 2007). This is notable, as lipophilic antioxidants generally have the potential for greater bioactivity than hydrophilic antioxidants, as lipophilic antioxidants can cross lipoprotein cell membranes more effectively (Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer, 2002). Total ORAC scores (sum of H-ORAC and L-ORAC) for all flours were greater than roughly 80% of the 277 foods and ingredients listed (USDA, 2007). Note that for the database when lipophilic data was not available, the total ORAC score was taken as the H-ORAC score (USDA, 2007).

3.2. ORAC of roasted peanut seed and blanched skins

To better understand roast effects in relation to antioxidant capacity of peanuts and peanut based ingredients, whole peanut seed were sequentially roasted at 166 °C from 0 to 77 min to obtain materials of different roast colour. Roasted samples were blanched, analytically extracted, and subsequently analysed for changes in

both H-ORAC and L-ORAC. The blanched peanut skins were also collected, analytically extracted, and subsequently analysed for changes in H-ORAC and L-ORAC as recent work has suggested the excellent potential for peanut skin to serve as a functional food ingredient (O'Keefe & Wang, 2006; Yu et al., 2006). A total of five time points were sampled between 0 and 77 min and the Hunter L -value, which gives an objective measure of lightness, was recorded for both the blanched peanut seed and skins. L -value of the blanched, roasted peanuts is interpreted as a relative measure of sugar–amino acid reactions and the development of melanoidin like compounds, i.e. Maillard browning, within roasted peanut seed (Ahmed & Young, 1982; Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2000). Work with model Maillard Browning systems, such as heated glucose solutions containing single amino acids, has demonstrated increased antioxidant capacity with colour change intensity, and this increase was generally ascribed to the formation of Maillard browning reaction products (Manzocco et al., 2000). Basing ORAC data on peanut seed (or skin) colour is also industrially relevant, as it is common industry practice to roast peanut products to specific colours depending on the desired product application.

3.2.1. H-ORAC of roasted peanut seed

For roasted, blanched seed, H-ORAC linearly ($R^2 = 0.91$) increased with decreasing L -value (Fig. 2A), suggesting that darker seed are consistently associated with greater H-ORAC. Previous work indicated that roasting at 175 °C/10 min resulted in peanuts with an L -value of approximately 50 and a subsequent increase in H-ORAC of approximately 16% for non high oleic peanuts (similar to current study) (Talcott et al., 2005b). As mentioned previously, peanuts roasted for the current study at 166 °C for 21.5 min had an L -value of 47.5 ± 1.0 with a corresponding increase in H-ORAC of roughly 14% (Figs. 1A, 3A), confirming earlier results. No decrease in H-ORAC was observed for more intense roast treatments up to 77 min at 166 °C, with small increases measured across the roast curve. The final seed sample collected at 77 min had an L -value of 28.9 ± 1.3 and while too dark for typical dry roast products, such roast intensities are utilised in the production of specialised peanut based ingredients, such as flavour extracts.

3.2.2. H-ORAC of roasted peanut skin

H-ORAC of peanut skins increased approximately 38% after roasting 7 min (H-ORAC = $209,710 \pm 13,030$; L -value = 40.8) compared

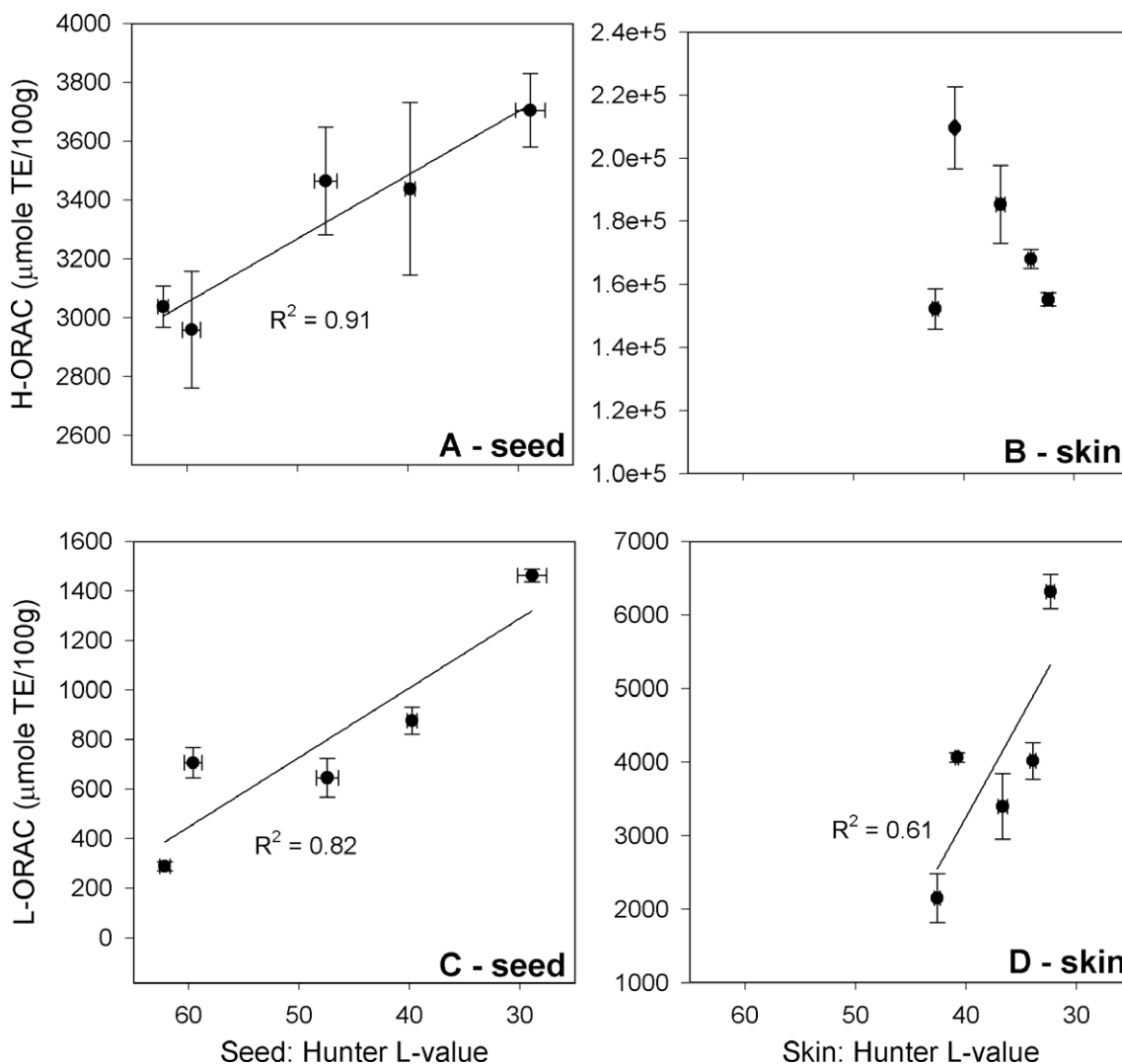


Fig. 2. Hydrophilic ORAC of roasted, blanched peanut seed (A) and the corresponding skins (B) as a function of Hunter *L*-value after roasting. Lipophilic ORAC of roasted, blanched peanut seed (C) and the corresponding skins (D) as a function of Hunter *L*-value after roasting. Data points are mean values and error bars are standard deviations. Solid lines are best linear fits.

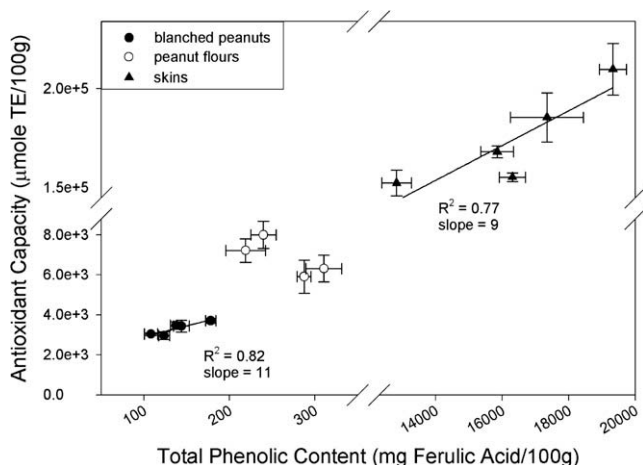


Fig. 3. Hydrophilic antioxidant capacities of roasted, blanched peanut seed, peanut flours, and skins as a function of total phenolic content within the extracts. Data points are mean values and error bars are standard deviations. Straight lines are linear best fits for the blanched peanuts and the collected skins.

to the value for the raw skins (H-ORAC = 152,290 ± 6,410; *L*-value = 42.6). However, beyond 7 min, H-ORAC declined with increasing roast, such that at 77 min (*L*-value = 32.4) H-ORAC was 155,220 ± 2090, a value similar to that of the non-roasted skins (Fig. 2B). The large increase in H-ORAC for skins after roasting 7 min is at least partially attributable to moisture loss upon roasting, as raw skins were not dried prior to testing. However, total moisture content of raw peanut skins was 11.0 ± 0.3%, indicating that the increase in H-ORAC at 7 min is attributable to more than simply moisture loss. Like whole peanut seed, peanut skins became progressively darker with increasing roast intensity across the 77 min timeframe (Fig. 2B). However, it is not clear if this darker colour is attributable to Maillard browning or a different phenomenon, or a combination of both. Peanut skins do contain 16–18% protein (Sobolev & Cole, 2004) but the sugar content is expected to be quite low, which should limit Maillard browning within the skins. As discussed next, peanut skins are established as rich source of phenolic compounds, hence the skin darkening observed with increasing roast could be a function of phenolic polymerisation. It is also considered that such rearrangements may limit these compounds effectiveness at interacting with assay components resulting in decreased antioxidant capacity scores, when in fact

antioxidant capacity may not be changed. It is notable that peanut skin colour was darker than the blanched seed for all time points except for the most extreme roast (77 min) at which the blanched seed were darker than the corresponding skins (Fig. 2).

3.2.3. Total phenolic content and H-ORAC comparison

Talcott et al. (2005b) found that the total phenolic content of peanut seed increased upon roasting at 175 °C/10 min, and this increase was associated with the corresponding increase in H-ORAC. Roasting was hypothesised to release phenolic compounds previously bound within the various cellular components of the peanut seed (Talcott et al., 2005b). To further test this theory, total phenolic content was determined for all hydrophilic extracts, including peanut flours, blanched seed and skins and compared to the corresponding H-ORAC values (Fig. 3). A linear correlation ($R^2 = 0.82$) was observed between H-ORAC and measurable total phenolic content for seed, indicating that as the seed were roasted to higher intensities, total phenolic content increased. Total phenolic content ranged from 108 ± 8.0 , 137 ± 7 and 178.0 ± 6 mg ferulic acid equivalents (FAE)/100 g seed for seed roasted at 0, 21.5 and 77 min respectively (Fig. 3). For comparison, gallic acid equivalents were also determined, and the calculated conversion value was 0.68 ferulic acid/gallic acid equivalents. From a practical perspective, it is important to note that the highest roast intensities utilised in this study were not detrimental to H-ORAC or total phenolic content of the peanut seed as compared to the raw sample (Figs. 2 and 3).

The linear correlation ($R^2 = 0.77$) for the plot of total phenolic content and H-ORAC of peanut skins further emphasised the relation between these two parameters (Fig. 3). Phenolic content in the skin initially increased from 0 to 7 min roast, with values of $12,830 \pm 450$ and $19,330 \pm 410$ mg FAE/100 g, respectively, an approximate 51% increase. Again, this is at least partially attributable to moisture loss upon roasting, as raw skins were not dried prior to testing. However, peanut skins were only 11% moisture, which suggests the initial heating at 166 °C for 7 min liberated phenolic compounds previously bound within the skin matrix, similar to the hypothesis suggested for whole peanut seed and roasting (Talcott et al., 2005b). Further roasting decreased phenolic content of the skin, such that at 21.5 min and 77 min, phenolic content was $17,350 \pm 1100$ and $16,310 \pm 390$ FAE/100 g, respectively. Hence, higher roast intensities seem to degrade phenolics within the skin but these values were still greater than that of raw skin. Slopes of best fit lines for H-ORAC vs. total phenolics for seed and skins were roughly 11 and 9, respectively; values that are similar, especially when considering H-ORAC and total phenolic content for the skins were roughly two orders of magnitude greater than that of seed or flours (Fig. 3). Catechin equivalents were also determined for skins as this class of compounds is more relevant to peanut skin chemistry, and the calculated value was 0.74 ferulic acid/catechin equivalents.

Previous work found roasting at 175 °C for 5 min decreased the total phenolic content of peanut skins by about 5% and correspondingly this roast treatment minimally affected the hydrophilic antioxidant activity (determined by an end point free radical scavenging method) of peanut skin (Yu et al., 2006). The roast conditions used in the previous study are most comparable to roasting at 166 °C for 7 min in the current study, after which skin H-ORAC increased approximately 38% (Fig. 2B). In the previous study, the authors freeze dried peanut skins collected from raw peanuts, whereas in the current study, skins from raw peanuts were used as is. Hence, the moisture difference between the raw and roasted skin samples were greater in the current study; however, as previously mentioned, moisture loss at 7 min roast was no more than 11%, whereas the increase in H-ORAC was approximately 38% (Fig. 2B). Therefore, the different observations in the studies for

antioxidant capacity of peanut skin after a mild roast treatment could be attributed to the different antioxidant assays used, different extractions conditions, different roast conditions, varietal differences in skin composition, release of phenolics or other factors.

3.2.4. L-ORAC of roasted peanut seed

L-ORAC for the blanched seed increased linearly ($R^2 = 0.82$) as the peanuts became darker with longer roast times (Fig. 2C). L-ORAC ranged from 290 ± 20 to 1460 ± 30 $\mu\text{mole TE}/100$ g for the raw and 77 min roasted seed, respectively (Fig. 2C). L-ORAC increased approximately 145% to 710 ± 60 $\mu\text{mole TE}/100$ g after roasting only 7 min (L -value = 59.6 ± 0.8), followed by a relative leveling until another increase for the final time point (L -values = 28.9 ± 1.3) (Fig. 2C). In contrast, significant ($P < 0.05$) changes in H-ORAC did not occur until 21.5 min of roast (L -value of 47.6), followed by slight increases at L -values ≤ 37.4 (Fig. 2A). This large increase in L-ORAC for peanut seed upon mild roasts (Fig. 2C) may explain why L-ORACs of light and dark roast peanut flours were greater than that of raw peanuts despite having much lower total oil contents (Fig. 1B). While reactive products generated during Maillard browning are well established to have increased water soluble antioxidant capacity (Manzocco et al., 2000), we are unaware of any studies directly measuring these types of antioxidant changes in a lipophilic environment. However, from data in Fig. 2C it seems Maillard reactant products are soluble in the lipophilic extracts prepared from roasted peanut seed and these compounds are in turn capable of imparting increased antioxidant capacity to these extracts.

3.2.5. L-ORAC of roasted peanut skins

L-ORAC for peanut skins also increased linearly ($R^2 = 0.61$) with increasing roast intensity (Fig. 2D). L-ORAC response for skin extracts as a function of roast was similar to the L-ORAC response for the blanched peanut seed (Fig. 2C and D). That is, there was a significant ($P < 0.05$) increase after 7 min roast (L -ORAC = 4060 ± 60) as compared to the raw skin sample (L -ORAC = 2150 ± 330), followed by a relative leveling until the final time point was measured at 77 min roast (L -value = 32.4) with an L-ORAC of 6320 ± 230 (Fig. 2D). From the data in Fig. 2D, it is hypothesised that the roasting process also increased the concentration of oil soluble antioxidants within the lipophilic skin extracts. Comparison to the USDA ORAC database found that the L-ORAC of all roasted peanut skins was greater than roughly 94% of the 164 food and ingredients listed (USDA, 2007). Furthermore, total-ORAC (sum of H-ORAC and L-ORAC) for peanut skins (7 min roast) was greater than all but 4 of 277 foods and ingredients listed (USDA, 2007). These data further reinforce both the excellent nutritional properties of peanut skin, and the excellent potential that industrially blanched peanut skins have to serve as the basis for nutraceutical ingredients. Others have made similar conclusions for skins of both almonds (Siriwardhana & Shahidi, 2002) and hazelnuts (Shahidi, Alasalvar, & Liyana-Pathirana, 2007) and this was generally attributed to the increased phenolic content of these materials.

3.3. Tocopherol content of roasted peanut seed and various lipophilic extracts

Mechanically pressed oils from the roasted, blanched seed, as well as the various hydrophobic extracts from all samples, i.e. seed, flours and skin, were analysed for Vitamin E content as a function of roast intensity to better understand L-ORAC data. Vitamin E is a blanket term that refers to naturally occurring antioxidants in edible oils that primarily consists of α , β , γ and δ tocopherols as well as the corresponding tocotrienols (Burton & Ingold, 1986; Franke, Murphy, Lacey, & Custer, 2007). All tocopherols are "chain

breaking” antioxidants meaning they prevent oxidation after the initiation phase of oxidation by “trapping” the generated free radicals (Burton & Ingold, 1986). α -Tocopherol is considered the most biologically active antioxidant of the tocopherols (Burton & Ingold, 1986). Representative chromatograms for oils mechanically expressed from roasted seed and directly solubilised in the HPLC solvent, as well as tocopherol standards, are presented in Fig. 4. Peak areas were converted to 100 g seed weight basis and average values for α , β , γ and δ tocopherol were 12.80 ± 0.73 , 0.65 ± 0.03 ,

10.20 ± 0.54 , and 0.78 ± 0.04 mg/100 g for raw peanut seed, respectively. By comparison, previous authors reported that α -tocopherol (α -T) ranged from 12.93 to 7.45 mg/100 g and γ -tocopherol (γ -T) ranged from 9.49 to 22.97 for five different varieties of raw, mature runner peanuts (similar to current peanuts) (Hashim, Koehler, & Eitenmiller, 1993). The first two peaks within all oil chromatograms had retention times of ~ 2.78 and 3.42 min, with the areas of these peaks remaining essentially constant with changing roast intensity (Fig. 4). The identities of these peaks are not known.

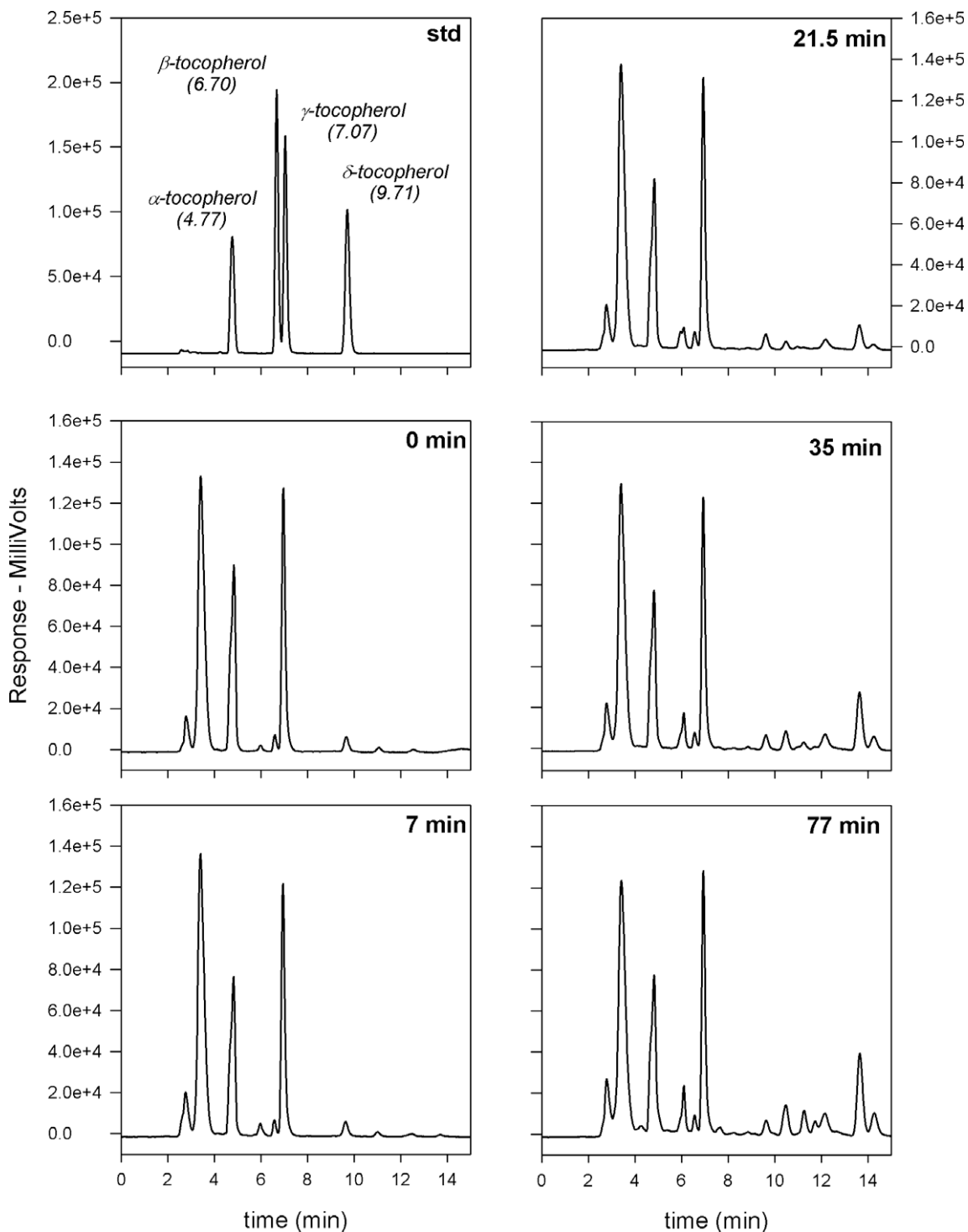


Fig. 4. Chromatograms of mechanically pressed oils from roasted peanut seed immediately after roasting. Symbols appear on the graph. Note the scale difference for standard compounds as compared to the expressed oils.

Within the chromatograms, there was an increase in peaks eluting after 10 min with increasing roast intensity, and the most prominent peak within this group had a retention time of ~ 13.78 min (Fig. 4). The steady increase in these peaks with increasing roast intensity suggests that these peaks may represent oil soluble Maillard reaction products. Comparison of equivalent chromatograms prepared from the lipophilic extracts of peanut flours revealed that there was also a notable increase in peaks eluting after 10 min for darker roast samples (data not shown). Work is ongoing to establish more definitive identifications for these compounds. Analysis of lipophilic skin extracts revealed non detectable levels of any tocopherol across the roast range (data not shown). Furthermore, no prominent peaks were detected under the current analytical conditions. Therefore, the high L-ORAC of lipophilic skin extracts is attributed to compounds other than Vitamin E or the unidentified peaks observed for the darker roasted peanut seed.

3.4. Tocopherol degradation in roasted oil under accelerated oxidative conditions

Roasting, as compared to raw peanuts, enhances the degradation of Vitamin E, specifically α -T and γ -T, in whole peanuts upon extended storage (Chun, Lee, & Eitenmiller, 2005). Roasting for this previous study was conducted in a dual zone oven at 135 °C/10 min followed by 190 °C/10 min to yield blanched seed with a Hunter L-value of 49 ± 1 (Chun et al., 2005). For the current work, Vitamin E degradation within peanuts roasted to a range of intensities was measured by placing 6 mL of the mechanically extracted oils within an incubator at 85 ± 3 °C in open beakers (surface area = ~ 12.57 mm²) to accelerate oxidation. Samples were periodically sampled for Vitamin E content and the data is summarised in Fig. 5. In both the current study and the work of Chun et al. (2005), α -T is seen to be more heat labile than γ -T (Fig. 5A and B). After 1 day of the accelerated oxidative conditions, the greatest decreases in α -T were observed in samples roasted for 7 and 21.5 min, whereas degradation of this antioxidant was substantially less with higher roast intensities (Fig. 5A). After 4 days of the accelerated oxidative conditions, α -T was undetectable for oils from seed roasted at either 7 min or 21.5 min; however, increasing roast intensity increasingly preserved α -T, such that at 77 min α -T levels were higher than that measured in the raw peanut sample (Fig. 5A). Levels of γ -T were minimally affected by all roast intensities after 1 day of accelerated storage (Fig. 5B). However, after 4 days, γ -T levels were lowest for samples roasted 7 min but increasing roast minimised γ -T degradation such that levels of this compound in samples roasted at 77 min were equivalent to that of

the raw peanut oil (Fig. 5B). Similar trends are noted for the sum of the tocopherols (Fig. 5C) as α -T and γ -T comprise the vast majority of total tocopherols within peanut oil. From this work, it seems the final concentration of Vitamin E in roasted peanuts or peanut oil is a balance between heat degradation and indirect heat stabilization via the formation of Maillard reaction products. This information may be applied to optimise Vitamin E levels in commercially roasted peanuts and peanut oils, especially darker roasted products.

3.5. H-ORAC of water soluble extracts prepared from hexane extracted peanuts roasted to different intensities

While currently not a commercial reality, the potential exists to use completely defatted, roasted peanut solids as a functional food ingredient. A primary advantage for such a product would be the relatively high protein content, which is expected to be approximately 50% w/w; however, the protein solubility for such a product would also be an important consideration for nutritional and functional applications. To this end, roasted, whole peanut seed were completely defatted and dispersed in deionized water at a final concentration of 5% w/w (see methods). After centrifugation, soluble portions were retained for further analyses. Peanut protein solubility is well established to be pH sensitive; however, for the current work, pH was unadjusted to better understand roast effects on this parameter. As seen in Table 1, pH of the aqueous soluble fractions steadily decreased with increasing roast intensity. By-products of the Maillard reaction are known to be acidic (Easa et al., 1996), which suggests these products are being solubilised in the current aqueous fractions. Protein solubility was substantially reduced at higher intensities such that there was an 85% reduction for protein extracted from defatted seed roasted at 77 min as compared to unroasted seed (Table 1). This agrees with earlier work showing that roasting decreases peanut protein solubility (Neucere, Ory, & Carney, 1969; Yu, Ahmedna, & Goktepe, 2007). Additionally, density of these aqueous solutions were measured at 23 °C to better understand total soluble material in these supernatants as a function of roast intensity, and again, density steadily decreased with increasing roast intensity (Table 1). H-ORAC generally declined with increasing roast under these solubility conditions (Table 1). A positive linear correlation ($R^2 = 0.96$) was observed in a plot of H-ORAC and soluble protein (plot not shown), meaning higher protein contents (less roast intensity) were associated with higher H-ORAC; however, two grouping of points at the extremes of this H-ORAC, protein relationship suggest finer differences in H-ORAC are likely associated with other factors.

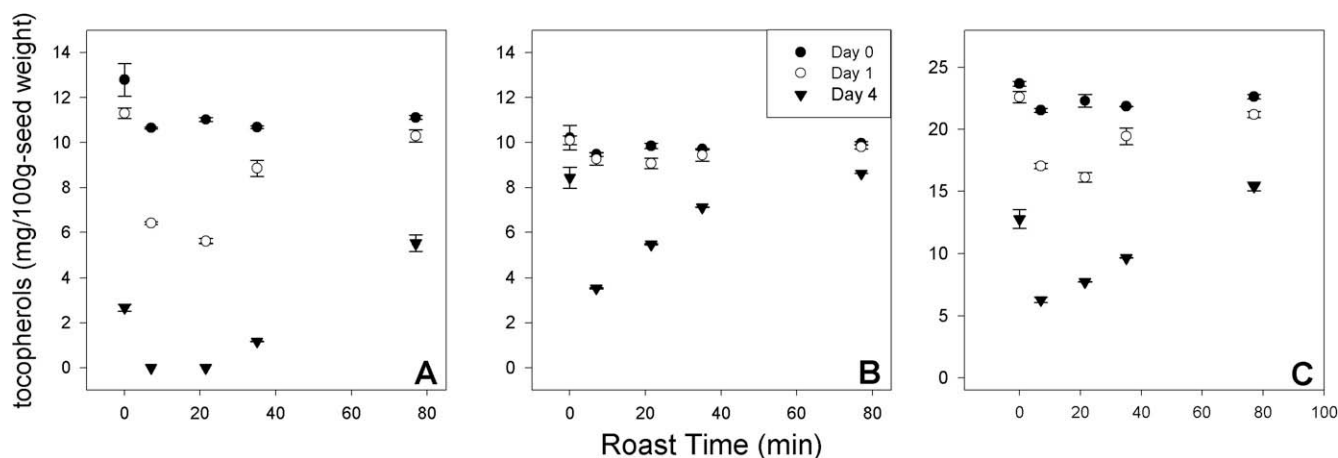


Fig. 5. Concentration of alpha- (A), gamma- (B) and total- (C) tocopherols as a function of roast intensity for mechanically pressed peanut oils stored under accelerated oxidative conditions at 85 °C. See text for experimental details.

Table 1

Properties of 5% water soluble fractions prepared from defatted, blanched peanut seed roasted at 166 °C for different times.

Roast time (min)	pH	Soluble protein (mg/ml)	Density-23 °C (g/ml)	H-ORAC (μmoles TE/100 g)
0	6.94	12.03 ± 0.34	1.0021 ± .0011	11640 ± 730
7	6.96	11.51 ± 0.20	1.0018 ± .0007	10840 ± 490
21.5	6.83	2.88 ± 0.18	0.9992 ± .0003	6780 ± 180
35	6.75	1.51 ± 0.05	0.9991 ± .0002	6830 ± 230
77	6.59	1.45 ± 0.02	0.9989 ± .0001	7360 ± 550

Previous work found that roasted and completely defatted peanut seed (180 °C/0–60 min) solubilised in water at a final protein concentration of 20 mg/mL displayed increased antioxidant properties as determined by a model linoleic acid emulsion system (Hwang, Shue, & Chang, 2001). These findings were attributed to the formation of Maillard reaction products, which as discussed earlier, have been associated with increased antioxidant capacity and increased peanut roast intensity. For the current work, aqueous samples were not normalised according to protein concentration, which as seen in Table 1, strongly correlates to the final antioxidant capacity of these water soluble extracts.

4. Conclusions

H-ORAC and L-ORAC of peanut flours, peanut seed, and peanut skins were all significantly ($P < 0.05$) affected by roasting. Darker roasts promoted greater H-ORAC and greater L-ORAC for both commercially available peanut flours and blanched peanut seed roasted at 166 °C, 0–77 min. These increases were attributed to greater concentrations of Maillard browning reaction compounds (measured indirectly by seed colour) and/or an increase in free phenolic compounds upon roasting. Total-ORACs for peanut skins (all roast levels tested) were roughly two orders of magnitude greater than those of peanut flours or seed. When compared to the USDA ORAC database, total ORAC of peanut skins (7 min) was greater than all but 4 of 277 foods and ingredients listed. As peanut skins are primarily a by-product of peanut processing, this data suggests that this low value material could readily function as a natural antioxidant in foods and should also be further investigated for potential nutraceutical applications. Measurements of Vitamin E within the roasted seed found that lighter to medium roasts promoted the most rapid degradation of α -T and γ -T within peanut oil upon accelerated oxidative conditions. However, degradation of these important lipophilic soluble antioxidants was significantly ($P < 0.05$) reduced in oils from peanuts roasted to darker colours, presumably by the corresponding concentration increase in lipid soluble Maillard reactant products.

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