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Effects of Pre-germination Treatment on the Phytate and Phenolic Contents of Almond Nuts

*Liang Lin[1], Freda Xin You Giam[1], Xin Min Foo[1], Nadia Marie Hui Lian Yeo[1], Charlene Jia Ling Koh[1], Nur Hatika Binte Sa'Aban[1], Wai Mun Loke[*1,2]*

[1] Food Science & Nutrition Group, School of Chemical & Life Sciences, Nanyang Polytechnic, Nanyang, Singapore

[2] Centre for Functional Food & Human Nutrition, School of Chemical & Life Sciences, Nanyang Polytechnic, Nanyang, Singapore

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Abstract

This study examined if pre-germination altered the water content and water activity, contents of phytate, total phenolic, (±)-catechin, quercetin and total antioxidant capacity of almond (*Prunus dulcis*) kernel. Raw almond kernels were submerged for 15 hours in water, 0.02 mol dm^{-3} phosphate buffer solution (pH 5.0) and 0.02 mol dm^{-3} phosphate buffer solution (pH 7.0) at 25 and 40ºC, respectively. The content and activity of water in the kernels before and after the pre-germination treatments were measured by oven drying and dew point water analysis, respectively. The total phenolic and phytic acid contents of the kernels were quantified by using Folin-Ciocalteu and a published spectrophotometric assay, respectively. (±)-Catechin and quercetin contents in the almond kernels were determined using gas-chromatography mass spectrometry. The total antioxidant capacity of the kernels were measured by 2,2'-diphenyl-1- picrylhydrazyl assay. Treatment with water, PBS pH 5 and PBS pH 7 significantly increased the water, total phenolic, (±)-catechin contents and total antioxidant capacity of the almond kernels regardless of the treatment temperatures (25 or 40°C). The phytic acid and quercetin contents were significantly elevated after the three treatments at 40°C. The total phenolic, (±)-catechin, quercetin and phytate contents in the almond kernels contributed significantly to its antioxidant property. Our results suggested that the phytochemical compositions of the almond kernels changed during pre-germination. The temperature and pH of the medium exert differential influence on the phytochemical compositions of the pre-germinated almond kernels.

Keywords: Almond kernels, Phytic acid, Pre-germination, Total antioxidant capacity, Total phenolic content.

Introduction

The kernels of nut trees contain significant amounts of phenolic compounds and therefore, may serve as a dietary source of phenolic compounds (Kornsteiner *et al*., 2006). These phenolic compounds are aromatic plant secondary metabolites containing the phenol moiety with molecular weights ranging from less than 100 Da to greater than 30,000 Da (Kammerer *et al.,* 2012), and have been extensively examined for potential health benefits (Kammerer *et al*., 2012). Phytic acid is a fiber-associated constituent found abundantly in plant (Reddy *et al.,* 1982). It contributes to about 1-5% by weight of edible legumes, cereals, oil seeds, and kernels (Cheryan & Rackis, 1980). Phytic acid is the major phosphorus storage compound in plant seeds, which contributes to about 80% of the phosphorus in plant seeds (Lopez *et al.,* 2002). During germination, phytase catalyzes the degradation of the phytate salt (Lopez *et al*., 2002)

[*]**Corresponding author**: Email: loke_wai_mun@nyp.edu.sg

and releases the phosphorus to be available for the growing seedling (Dost & Tokul, 2006).

Pre-germination of grains has been reported to lower their phytic acid contents (Shallan *et al*., 2010). The same process also increased total phenolic contents in these grains (Gujral *et al.,* 2012). Traditionally, grain pre-germination was performed by submersion in water for a designated time period. The time period and the osmosity of the soaking solution determined the texture and composition of the soaked product (Shallan *et al*., 2010). Pre-germination of tree kernels in water is believed to exert similar effects observed with that of the rice grains, though scientific evidence is currently absent.

This study aimed to examine if the pre-germination processes altered the water contents and water activities, contents of total phenolic, phytic acid, (±)-catechin, quercetin contents in almond kernels. The same study also aimed to compare the total antioxidant capacity of the almond kernels before and after the pre-germination process. We hypothesised that the phenolic and phytic acid contents in the almond kernels are modified by pre-germination. These effects differ depending on the temperature and pH of the treatment medium. We also hypothesised that the changes in phenolic and phytic acid contents are associated with the changes in total antioxidant capacity in almond kernels.

Materials and Methods

Chemicals & Materials

Raw almond (*Prunus dulcis*) kernel was purchased from a local supermarket. Methanol, gallic acid, Folin-Ciocalteu's phenol reagent, sodium chloride, sodium carbonate, sodium phytate, hydrochloric acid, ammonium iron (III) sulfate.12H_2O, 2,2'-bipyridine, thioglycollic acid, 2,2-diphenyl-1- picrylhydrazyl (DPPH•), ascorbic acid, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), ascorbic acid and phosphate buffered saline (PBS) were purchased from Merck (NJ, USA). 2,3,4,5,6-pentafluorophenylbromide (PFPBr), N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA), pyridine, isooctane, (±)-catechin, quercetin were purchased from Sigma Aldrich (St Louis, MO). All experiments and chemical analyses were conducted in the investigator's laboratory.

Pre-germination of almond kernels

Raw almond kernels (100g) were completely submerged at 25ºC for 15 hours in distilled water (pH 7.0), 0.02 mol dm^{-3} phosphate buffered solution (pH 5.0) and 0.02 mol dm^{-3} phosphate buffered solution (pH 7.0), respectively. The same pre-germination experiments were repeated at 40ºC. The treated kernels were rinsed with distilled water and dried at 40 ºC for 24 hours in an conventional oven before chemical analysis. All chemical analyses were performed within one month after the pre-germination treatment.

Water content and activity

Almond kernels were freshly grounded using a blender (Blendtec, Model No. HP3A) on the day of measurement. The water contents in the raw and soaked kernels were analysed using the conventional oven drying method [AOAC Official Method 925.40]. The water activity was measured using a dew point water activity meter (AquaLab, Model No. 4TE). All measurements were conducted in triplicates.

Determination of total phenolic content and quercetin

Total phenolic content in the kernel extract was quantified by using Folin-Ciocalteu assay (Actis-Goretta *et al.,* 2006). Briefly, freshly grounded kernels (~0.5 g) were weighed and extracted with 80% aqueous methanol (50 mL) in an ultrasonic bath (WiseClean, Model No. WUV-D22H) at room temperature for 20 minutes. The supernatant, obtained after centrifuging the kernel mixture at 3000 rpm for 5 mikerneles at 5ºC, was assayed for its total phenolic content. The total phenolic content in each test sample

was quantitated against standard gallic acid solutions and was expressed as mg gallic acid equivalents (GAE)/100g fresh weight (Swain & Hillis, 1959).

The amounts of (±)-catechin and quercetin in the almond kernel extract were determined by a published method using Gas Chromatography Mass Spectrometry (GC-MS)(Fuzfai & Molnar-Perl, 2007). The kernel extract was subjected to acidic hydrolysis in 1.0 mol dm-3 methanolic HCl at 80°C for 30 mikerneles. The hydrolysed mixture was extracted twice with ethyl acetate (1 mL), dried under nitrogen, and derivatized with BSTFA (100 µL) and pyridine (50 µL) at 40°C for 60 minutes. The trimethylsilyl (TMS) derivatives were analyzed on an Agilent 6890 gas chromatograph coupled to a 5973 mass spectrometer with the use of a cross-linked methyl silicone column (25 m x 0.20 mm, 0.33-mm film thickness, HP5-MS). Aliquots (1.0 µL) were injected in the splitless mode. The column temperature was held at 150°C for 1 mikernele and then increased to 300 °C at a rate of 20 °C/min and to 320°C at a rate of 30 °C/min. Helium (0.7 mL/min) was used as the carrier gas. Peak identification was based on retention time, and the mass spectra were compared with authentic standards (±)-catechin and quercetin). Quantification was performed by using calibration curves obtained from authentic standards. The (±)-catechin and quercetin contents were expressed as the mass of quercetin in 100 g of dry almond kernels.

Determination of phytic acid content

Freshly ground kernels (~2 g) was weighed and extracted with 0.2 mol dm^{-3} hydrochloric acid (15 mL). The supernatant was obtained by centrifuging the mixture at 3000rpm for 5 mikerneles at 5ºC (Eppendorf, Model No. 5810). The phytic acid content in the kernel extract was quantified using a published spectrophotometric method (Haug & Lantzsch, 1983). The phytic acid content was calculated from the calibration curve and was expressed as mg/g of fresh weight eight.

Measurement of total antioxidant capacity

The total antioxidant capacity of the kernels were measured by 2,2'-diphenyl-1- picrylhydrazyl (DPPH•) assay (Villano *et al.*, 2007). The total antioxidant capacity was expressed as the equivalence of the ascorbic acid concentration per gram of the kernel.

Statistical analyses

Statistical analyses were performed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). Data were presented as mean ± standard deviation. Group differences for two and more than two groups were analysed using unpaired *t*-tests and ANOVA with Tukey's post-hoc adjustment, respectively. Correlation analysis was performed using Pearson's correlation. Significance difference and correlation were marked when $p < 0.05$.

Results

Treatment with water, PBS pH 5 and PBS pH 7 at 25°C significantly increased the water contents, total phenolic contents, (±)-catechin contents and total antioxidant capacity of the almond kernels ($p<0.05$ using ANOVA with Tukey's post-hoc adjustment, Fig. 1). The observed augmentation of water contents, total phenolic, (±)-catechin contents, and antioxidant capacity did not differ between the three treatments (Fig. 1). Quercetin contents in the almond kernels were significantly elevated after treatment with PBS at pH 5 and pH 7 but not with distilled water at 25°C (Fig. 1e). Water activity and phytic acid contents were unaffected by the three treatments at 25°C (Fig. 1c & 1f).

The water, total phenolic, (±)-catechin, quercetin and phytic acid contents, and total antioxidant capacity of the almond kernels were significantly elevated after the three treatments (distilled water, PBS pH 5 and PBS pH 7) at 40°C ($p<0.05$ using ANOVA with Tukey's post-hoc adjustment, Fig. 1). Water activity was not affected by the treatments (Fig. 1b). The total phenolic contents and total

antioxidant capacity of the almond kernels were significantly modified at 40 °C in the order: water < PBS pH 5 < PBS pH 7 (Fig. 1c & 1g). The treatments significantly affected the (±)-catechin contents in the order: deionised water < PBS pH 7 < PBS pH 5 (Fig. 1d) but did not differ in their effects on quercetin contents (Fig. 1e). Treatment with PBS pH 7 significantly augmented the phytic acid content when compared to PBS pH 5 and water treatments (Fig. 1f).

An increase in the treatment temperature did not produce significant changes in water contents, water activity, total phenolic, (±)-catechin, and total antioxidant capacity of the almond kernels (p<0.05 using unpaired t-test, Fig. 1). Phytic acid contents were significantly elevated in the almond kernels after treatments with distilled water, PBS pH 5 or PBS pH 7 at a higher temperature (40°C) when compared to the same treatments at a lower temperature (25°C) (p<0.05 using unpaired t-test, Fig. 1f).

The total antioxidant capacity of the almond kernels significantly correlated to their phytic acid, total phenolic, (±)-catechin and quercetin contents (phytate, r = 0.754, p < 0.05; total phenolic, r = 0.625, p < 0.05; (±)-catechin, r = 0.651, p < 0.005 and quercetin, r = 0. 648, p < 0.005).

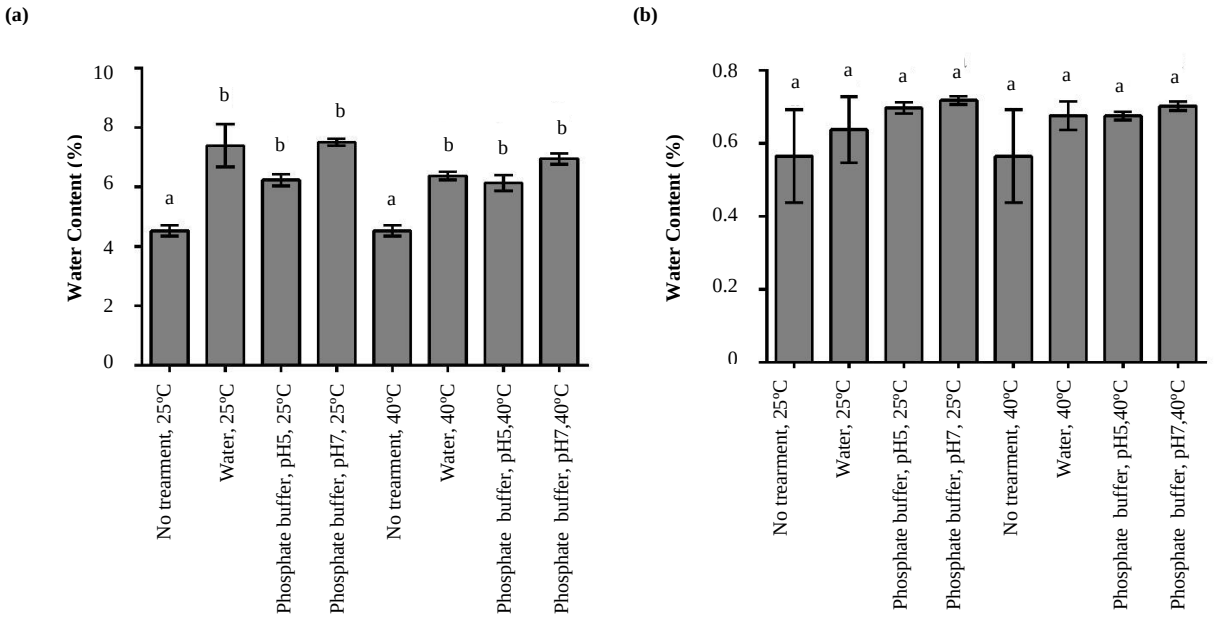

Fig. 1. (a) Water content (%), (b) water activity.
[a,b,c,d] Values with different superscripts are significantly different to one another using ANOVA with Tukey's post-hoc adjustment. * p < 0.05 compared to the value at temperature of 25°C using unpaired t-test.

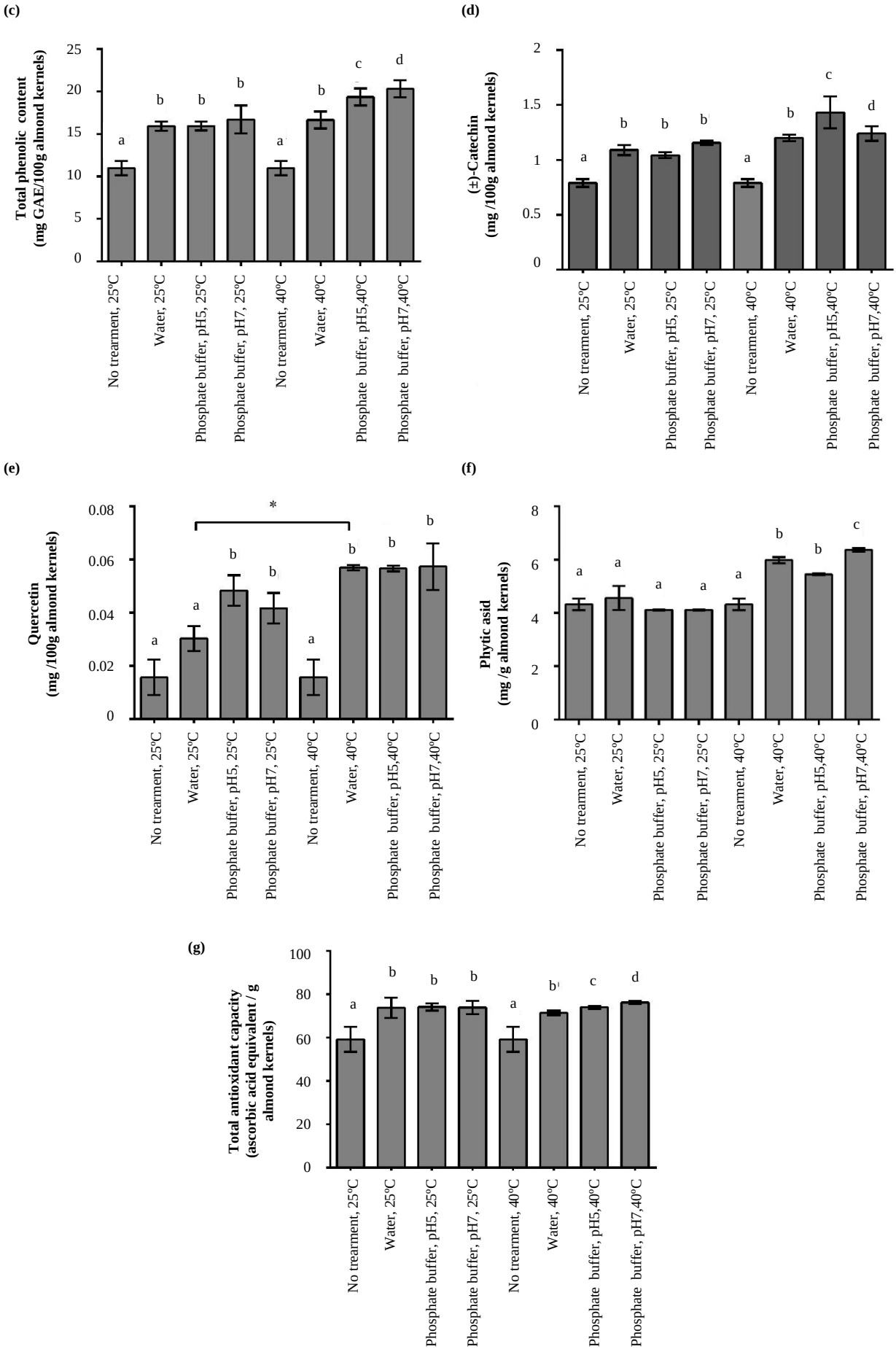

Fig. 1. Continued. (c) total phenolic (mg gallic acid equivalent/ 100g nut), (d) (±)-catechin (mg/ 100g nuts), (e) quercetin (mg/ 100g nuts), (f) phytic acid content (mg/ g nut), and (g) total antioxidant capacity (ascorbic acid equivalent/ g nut) of almond kernels before and after treatment in water, 0.02 mol dm^{-3} phosphate buffer saline pH 5.0 or 0.02 mol dm^{-3} phosphate buffer saline pH 5.0 at temperature of 25°C or 40°C.

Discussion

Previous reports suggested that the temperature of the pre-germination process may affect the chemical compositions of the grains (Carlson & Poulsen, 2003; Lestienne *et al*., 2005; Liang *et al*., 2008). Our study examined the effects of pre-germination on tree nut kernels. Our results showed that the pre-germination temperatures did not significantly change the water content, water activity, total phenolic, (±)-catechin, quercetin contents and total antioxidant capacity of the almond kernels. A higher pre-germination temperature increased the phytic acid contents of the almond kernels. The pH of the pre-germination medium had a great influence on the phenolic and phytate contents of the almond kernels. More experiments are required to elucidate the mechanisms by which the temperatures and pH effect these composition changes in the almond kernels.

Phytic acid has been regarded as an anti-nutrient (Cheryan & Rackis, 1980) because of its strong ability to complex multi-charged metal ions, especially Zn(II), Ca(II) and Fe(III) (Harland & Morris, 1995). The high negatively charged phosphorylated structure of phytic acid binds with many divalent cations, proteins and starches, resulting in the malabsorption and the potential subsequent deficiency of these nutrients when a large quantity of phytic acid is consumed (Brune *et al*., 1989; Zhou & Erdman Jr, 1995). The amount of phytic acid in rice grains was shown to be reduced significantly with prolonged soaking in water for three and 24 hours, respectively (Shallan *et al*., 2010). Larsson and Sandberg (Larsson & Sandberg, 1995) found that phytate can be hydrolysed by activating the endogenous phytase during food processes such as soaking, germination and fermentation. The reduction of phytate in rice grains by the process of soaking was reported to be influenced by the temperature and duration of treatment (Carlson & Poulsen, 2003; Lestienne *et al*., 2005; Liang *et al*., 2008). Contrary to the previous findings, we reported significant increases in phytic acid contents of the almond kernels after the pre-germination treatments. Our results with the almonds kernels were in agreeance with the current literature that the change in phytic acid content is influenced by the temperature and pH of the treatment medium. Additional studies are required to elucidate the possible biochemical reactions involved in the modulation of phytic acid content during the pre-germination of almond kernels.

The augmented total phenolic contents in treated almond kernels could be explained as an increase in the released phenolic compounds from the breakdown of the cell wall during the soaking processes. Insoluble phenolic compounds have been found to be cell wall components (Shibuya, 1984), which are bound to polysaccharides in the cell wall. Germination-induced saccharolytic enzymes break down endosperm and release bound phenolic compounds (Tian *et al*., 2004). Long-term germination increased the total phenolic content in legumes (Lin & Lai, 2006) and barley cultivars (Sharma & Gujral, 2010). A longer treatment may significantly increase the total phenolic contents in the almond kernels, though the lengthier soaking process would result in a significant degradation of the kernel endosperm. The total antioxidant capacity of almond kernels, as measured by the DPPH assay, were significantly augmented after treating with the different pre-germination media of the two temperatures used in this study. Barley showed a significant increase in the total antioxidant activity after germination as measured by DPPH radical scavenging assay (Sharma & Gujral, 2010).

Tree nut kernels, like almonds, have been shown to exert antioxidant properties though the specific molecules responsible were still unknown. The significant correlations between the total antioxidant capacity, phytate, total phenolic, (±)-catechin and quercetin contents suggested that the phytic acid and phenolic compounds in the almond kernels contributed significantly to its antioxidant property. Phenolic compounds may act as antioxidants by

donating a hydrogen atom to a free radical from their aromatic hydroxyl (OH) group and the resulting aromatic compound is able to support an unpaired electron as a result of the delocalisation of the electron system (Duthie & Crozier, 2000). Coincidentally, as a strong ion chelator, phytic acid inhibits the iron-catalysed formation of hydroxyl radical (Graf *et al*., 1987). Phytic acid's antioxidant capacity renders it a unique and versatile food preservative commonly added to meats, fishmeat paste, canned seafood, fruits, vegetables, cheese, noodles, soy sauce, juices, bread and alcoholic beverages (Dost & Tokul, 2006). Its antioxidant effect prevents product discolouration, increases nutritional quality and prolongs the shelf life of these products (Dost & Tokul, 2006). Phytic acid and phenolic compounds may act synergistically to exert antioxidant actions. More studies would be required to ascertain if the pre-germinated almond kernel exerts antioxidant actions in vivo, and if phytic acid should be regarded as an antioxidant or anti-nutrient.

Conclusions

Pre-germination altered the water, phytic acid, (±)-catechin, quercetin, total phenolic contents in almond kernels. The phytate, (±)-catechin, quercetin and total phenolic contents in the almond kernels contributed significantly to its antioxidant property. The temperature and pH of the medium exert differential influence on the phytochemical compositions of the pre-germinated almond kernels. Pre-germination conditions, such as treatment medium pH and temperature, should be optimized to better enhance the nutritive properties of almond kernels.

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