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ScienceDirect
LWT 40 (2007) 893–899
LWT
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Lipoxygenase activity in walnuts and almonds

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Received 8 September 2005; received in revised form 21 April 2006; accepted 3 May 2006

Abstract

The objective of this experiment was to investigate lipoxygenase (LOX) activity in walnut or almond homogenates. Walnut or almond kernels were heated with hot air at 55 °C for 2 or 10 min, or 60 °C for 2 or 10 min. The homogenates of untreated or heat treated walnut kernels exhibited greater LOX activity than the homogenates of untreated or heat treated almond kernels. Short-time heat treatments of 55 °C for 2 min or greater reduce LOX activity, retard the development of oxidative rancidity, and extend the shelf-life of walnuts and almonds during distribution and storage. Short-time heat treatments of walnut or almond kernels designed to control insect pests for international trade did not promote rancidity when compared to untreated walnuts or almonds.

Keywords: Heat; Rancidity; Lipoxygenase; Walnuts; Almonds; Pests

1. Introduction

A major problem in the storage and marketing of nuts is the infestation of insect pests. The tree nut industry relies heavily on fumigation with methyl bromide (MeBr) and hydrogen phosphine for postharvest insect control (Carpenter, Gianessi, & Lynch, 2000). However, regulatory actions against both MeBr and hydrogen phosphine may make these fumigants difficult to source or even unavailable to the industry. Owing to the uncertain future for chemical fumigation and public concern over residues in treated products, there has been a great interest in developing nonchemical treatments, in particular thermal treatments. Wang, Tang, Johnson, Mitcham, and Hansen (2002) proposed heat treatments based on radio frequency (RF) energy to control field and storage insect pests in in-shell walnut. Wang, Tang, Johnsosn, and Hansen (2002) demonstrated that a short-time heat treatment (55 °C for 5–10 min) did not promote rancidity in the treated walnuts. Buranasompob, Swanson, Tang, and Mao (2003) also reported that short-time heat treatments of walnut or almond kernels heated at 55 °C for 2 or 10 min, or 60 °C for 2 or 10 min did not increase rancidity when compared to untreated control walnut or almond kernels.

Walnut and almond kernels contain substantial quantities of triacylglycerols and polyunsaturated fatty acids, and thus are susceptible to oxidative and hydrolytic rancidity (Watkins, 2005). We hypothesized that short-time heat treatments inactivate lipoxygenase (LOX) or lipase enzymes and extend the shelf-lives of walnut and almond kernels.

LOX is a constituent of a wide variety of plants, particularly legumes, peas, beans, and peanuts (Whitaker, 1991). LOX (EC 1.13.11.12, linoleate:oxygen oxidoreductase) is an iron-containing dioxygenase that catalyses the oxidation of polyunsaturated fatty acids containing *cis*, *cis*-1,4-pentadiene units ($-CH=CH-CH_2-CH=CH-$) to produce conjugated unsaturated fatty acid hydroperoxides (Robinson, Zecai, Claire, & Rod, 1995). The naturally occurring polyunsaturated fatty acids linoleic, linolenic, and arachidonic acids contain one or more *cis,cis* penta-1,4-diene units. The occurrence and mode of action of LOX are reviewed by (Gardner, 1991; O'Conner & O'Brien, 1991; Whitaker, 1991). McCurdy, Nagel, and Swanson (1983) reported that LOX in dry pinto beans lost 100% activity after 15 s exposure to 100 °C, and 93% of the initial activity after a 10 min exposure to 65 °C.

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0023-6438/$30.00
doi:10.1016/j.lwt.2006.05.003

Zacheo, Cappello, Gallo, Santino, and Capello (2000) reported that LOX activity of the crude extracts of almonds was lost after 10 min exposure to 80 °C.

Macrae, Robinson, and Sadler (1993) and Young and Cunningham (1991) stated that almonds and almond products exhibit a longer shelf-life compared to other nuts because almonds contain smaller concentrations of polyunsaturated fatty acids and larger concentrations of α-tocopherol antioxidants. Almond kernels contain greater concentrations of α-tocopherol (~24 mg/100 g) than walnut kernels (~2.62 mg/100 g) (USDA, 1984; Watkins, 2005). Zacheo et al. (2000) reported that α-tocopherol retards lipid oxidation and extends the shelf-life of almonds.

The objectives of this research were to:

(1) study LOX activity in the homogenates of untreated and heat treated walnut and almond kernels; and
(2) study LOX activity of soybean LOX and soybean LOX added to the homogenates of untreated and heat treated walnut and almond kernels to assess antioxidant activity in the homogenates of walnut and almond kernels.

2. Materials and methods

2.1. Walnuts and almonds

Shelled walnuts, *Juglans regia* (cv. Chandler), were harvested in September 1998, and obtained from Quality Nut Company (Empire, CA, USA). Shelled almonds, *Prunus dulcis* (cv. Nonpareil) were harvested in August 1998 and obtained from Paramount Farms (Bakersfield, CA, USA). Shelled walnuts and almonds were stored at recommended optimum storage conditions of 2–4 °C (36–40 °F) in polyethylene bags before conducting the analyses.

2.2. Short-time heat treatments

The experiment was divided into four heating treatments. Heating treatments were 55 °C for 2 min, 55 °C for 10 min, 60 °C for 2 min, or 60 °C for 10 min, as predetermined, to deinfest unshelled walnuts and almonds (Johnson, Valero, Wang, & Tang, 2004; Wang, Tang, Johnson, & Mitcham, 2002; Wang, Tang, Johnson, & Hansen, 2002). Heating treatments were performed in duplicate on two replicates. After the heat treatments, the walnut and almond kernels were held at −25 °C until analysed. The conditions for heat treatments are presented in Buranasompob (2001).

2.3. Preparation of homogenates of walnut or almond kernels

Walnut or almond kernels were ground in a coffee bean grinder (Braun, Woburn, MA, USA) for 1 min. One hundred grams of ground kernels were blended with 200 ml of deionized water in a Waring blender for 1 min. The homogenates of walnut or almond kernels were held on ice until analysed. LOX activity of the crude aqueous extract of blended walnut or almond kernels were determined under standard assay conditions (pH 7.0, $T = 20$ °C) described herein.

2.4. Preparation of linoleic substrate and buffers

Linoleic acid (99%) (*cis*-9, *cis*-12-octadecadienoic acid), Bis-Tris buffer, Tris (hydroxymethyl) aminomethane buffer (Trizma® Base), sodium hydroxide, and hydrochloric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Linoleic acid substrate stock solution was prepared daily by pipetting linoleic acid (0.4 ml) into 5 ml of 1 N sodium hydroxide, adding deionized water to a volume of 500 ml, and agitating with a magnetic stirrer until the linoleic acid dispersed and the solution was clear (~30 min). The linoleic acid substrate stock solution was stored in an amber flask and refrigerated until used. Bis-Tris buffer was used to prepare buffer solutions at pH 5.0, 6.4, and 7.0. Tris-aminomethane was used to prepare 0.1 M buffer solutions at pH 9.0 by dissolving Bis-Tris or Tris-aminomethane buffer in 300 ml of deionized water in a 500 ml beaker. Deionized water was added to bring the buffer solution to 500 ml. A pH meter was used to determine the pH after bringing the buffers to a volume of 500 ml. The buffers were pH adjusted by the addition of 1 N HCl solution while stirring with a magnetic stirrer until pH of 5.0, 6.4, 7.0, and 9.0 were obtained. LOX activity of the homogenates of walnut or almond kernels were assayed at pH of 5.0, 6.4, 7.0 or 9.0.

2.5. Lipoxygenase assay equipment and procedures

A bench-top instrument developed at Washington State University (Reyes de Corcuera, 1998) was used in the determination of LOX activity using an oxygen electrode (Diamond General Co., Ann Harbor, MI, USA) to quantitate the rate of oxygen consumption. LOX activity was calculated as rate of change in concentration of dissolved oxygen in a reaction beaker and expressed as μM O_2/1 s. LOX activities were determined at pH 7.0.

Four grams of homogenate containing two grams of walnut or almond kernels and two grams of deionized water were weighed into a 20 ml beaker. Eight millilitres of linoleic acid substrate and 8 ml of the Bis-Tris buffer were injected into the reaction beaker with separate syringes. The reaction mixture was stirred continuously with an automatic stirrer in the reaction beaker during a 2 min assay at 20 °C. LOX activity is expressed as μM oxygen consumed per liter of reaction mixture of diluted walnut or almond kernel homogenates in a reaction beaker (20 ml) per second.

LOX activity of soybean LOX and soybean LOX in the homogenates of walnut or almond kernels were determined to assess antioxidant activity of shelled walnut and almond

kernels. Fifty milligrams of soybean LOX was dispersed in 100 ml of deionized water. The blank soybean LOX solution (1 ml) was placed in a 20 ml beaker and 4 ml of deionized water added. Eight millilitres of linoleic substrate and 8 ml of Bis-Tris buffer (pH 7.0 optimum pH for walnuts or almonds LOX) were injected into the reaction beaker with separate syringes. This reaction mixture was used as a blank. The reaction mixture was stirred continuously with an automatic stirrer in the reaction beaker during a 2 min assay at 20 °C.

One milliliter of soybean LOX solution was added to 4 ml of the homogenates of walnut or almond kernels to assess antioxidant activity of shelled walnut or almond kernels. Eight milliliters of linoleic substrate and 8 ml of selected buffer were injected into the reaction beaker with separate syringes. The reaction mixture was stirred continuously with an automatic stirrer in the reaction beaker during a 2 min assay at 20 °C.

Inhibition of soybean LOX by nut homogenates were calculated with Eq. (1):

$$\text{Inhibition of LOX activity (in per cent)} = \frac{(A + A_S) - A_M}{A + A_S} \times 100\%, \quad (1)$$

where A is the activity of the homogenates of walnut or almond kernels (0.1 g/ml), A_S the activity of soybean LOX (0.025 g/ml), and A_M the activity of the homogenate; activity of soybean LOX (0.025 g/ml) in the homogenates of walnut or almond kernels (0.1 g/ml).

2.6. Statistical analysis

The experiments were conducted in duplicate with two replications of each heating treatment. The general linear model procedure (SAS, 1989) was used to determine significant differences ($P \leqslant 0.05$) among LOX activity in the homogenates of untreated and short-time heat-treated walnut and almond kernels.

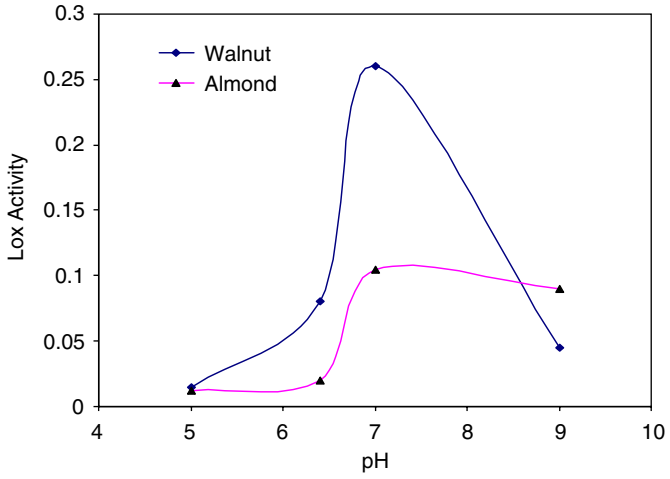

Fig. 1. Lipoxygenase activity (μM O_2/l s) in the homogenates of untreated almond or walnut kernels (1.0 g/ml) in pH range of 5.0–9.0. (—◆—) Walnut; (—▲—) Almond.

3. Results and discussion

3.1. Lipoxygenase activity of untreated walnut and almond homogenates

LOX activity in the homogenates of untreated walnut and almond kernels were investigated at pH 5.0, 6.4, 7.0, and 9.0 (Fig. 1). The homogenates of untreated walnut kernels exhibited the greatest LOX activity at pH 7.0. Less LOX activity was detected in walnut homogenates at pH 5.0 and 9.0 (Fig. 1). The homogenates of untreated almond kernels exhibited the greatest LOX activity at pH 7.0, and less LOX activity at pH 5.0 and 9.0 (Fig. 2). LOX in plants

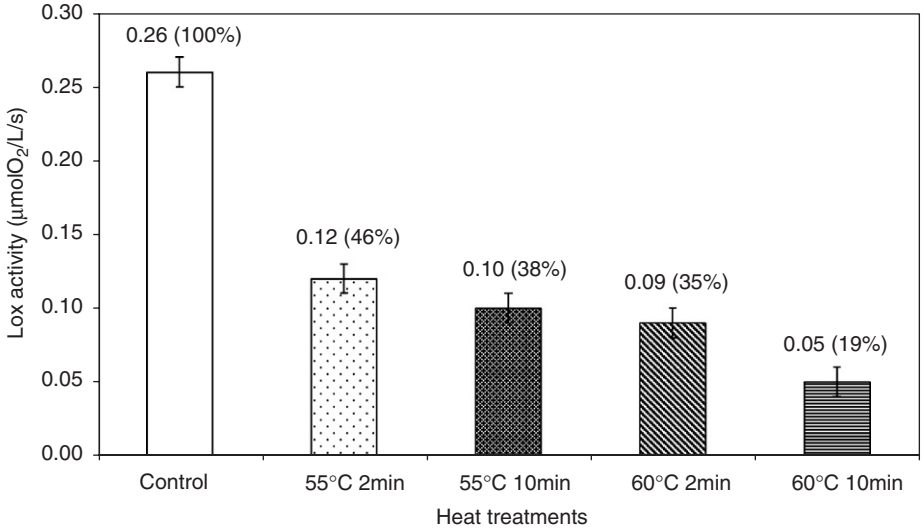

Fig. 2. Lipoxygenase activity in homogenates (0.1 g/ml @ pH 7.0) of control and heat treated walnut kernels.

exhibit optimum activity in the range of pH 5.5–7.5 (Romero & Barrett, 1997).

3.2. Homogenates of untreated and heat-treated walnut kernels

Fig. 2 presents LOX activity in the homogenate of untreated and walnut kernels heated at 55 °C for 2 or 10 min, or at 60 °C for 2 or 10 min. The initial mean LOX activity of the homogenates of untreated walnut kernels was 0.26 μM O_2/l s. The initial mean LOX activity of the homogenates of walnut kernels heated at 55 °C for 2 or 10 min, or 60 °C for 2 or 10 min were 0.12, 0.10, 0.09, or 0.05 μM O_2/l s, respectively (Fig. 2). Heating walnut kernels at 55 °C for 2 or 10 min, or 60 °C for 2 or 10 min inactivated 54%, 62%, 65%, or 81% of initial LOX activity, respectively. The homogenates of untreated walnut kernels exhibited significantly greater LOX activity ($P \leqslant 0.05$) compared to the LOX activity of the homogenates of short-time heat-treated walnut kernels.

Branch, Worthington, Roth, Chinnan and Nakayama (1987) reported that peanuts lost 46% LOX activity after a 90 s exposure to 79 °C. Branch et al. (1987) reported that oil extracted from untreated peanuts exhibited higher peroxide and fatty acid values than oils extracted from heat-treated peanuts. Heat-treated peanuts at 79 °C for 90 s exhibited a longer shelf-life than untreated peanuts (Branch et al. 1987). Buranasompob (2001) demonstrated that oils extracted from untreated walnut kernels exhibited significantly ($P \leqslant 0.05$) greater peroxide values than oils extracted from short-time heat-treated walnut kernels.

Buranasompob (2001) also demonstrated that short-time heat treatments at 55 or 60 °C for 2 or 10 min do not enhance development of oxidative rancidity during accelerated storage of walnut kernels (Wang, Tang, Johnson, & Mitcham 2002; Buranasompob et al., 2003). Henderson, Blank, and Sustackova (1991) reported that LOX in pea flour lost 100% activity after 25 min exposure to 65 or 70 °C, and after 15 min exposure to 80 °C. Williams, Lim, Chen, Pangborn, and Whitaker (1986) reported that LOX in English green peas lost 70% activity after a 10–15 min exposure to 60 °C.

Kermasha and Metche (1987) reported that French beans lost almost 100% LOX activity after a 150 s exposure to 96 °C. Kermasha and Metche (1987) reported that air-drying is an effective method to inactivate LOX enzymes, and suggested that oxidation of the unsaturated fatty acids in dried stored French beans may result from autooxidation rather than enzymatic activity. Buranasompob (2001) demonstrated that oils extracted from walnut kernels exhibited similar fatty acid values in both untreated and short-time heat-treated walnut kernels. Lipase activity was not detected in untreated control walnut kernels.

3.3. Homogenates of untreated and heat-treated almond kernels

Fig. 3 presents LOX activity in the homogenates of untreated and almond kernels heated at 55 °C for 2 or 10 min, or at 60 °C for 2 or 10 min. The initial mean LOX activity of the homogenates of untreated almond kernels was 0.11 μM O_2/l s. The initial mean LOX activity of the homogenates of almond kernels heated at 55 °C for 2 or 10 min, or 60 °C for 2 or 10 min were 0.09, 0.03, 0.09, and 0.03 μM O_2/l s, respectively (Fig. 3). Heating almond kernels at 55 °C for 2 or 10 min, or 60 °C for 2 or 10 min inactivated 18%, 73%, 18%, or 73% of initial LOX activity, respectively. The homogenates of untreated almond kernels exhibited significantly greater LOX activity ($P \leqslant 0.05$) than the homogenates of almond kernels heated at 55 or 60 °C for 10 min. No significant differences in LOX activity ($P > 0.05$) were observed between the homogenates of untreated almond kernels and the homogenates of short-time heat-treated almond kernels heat treated at 55 and

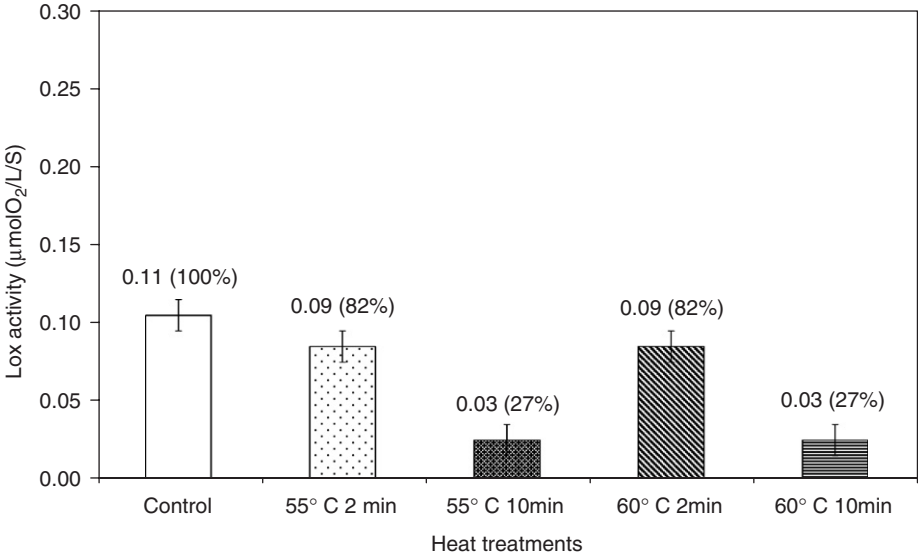

Fig. 3. Lipoxygenase activity in homogenates (0.1 g/ml @ pH 7.0) of control and heat-treated almond kernels.

60 °C for 2 min. No significant differences in LOX activity ($P > 0.05$) were observed between homogenates of almond kernels heat treated at 55 or 60 °C at equivalent heating times. Significant differences in LOX activity ($P \leqslant 0.05$) were observed between heating time of 2 and 10 min (Fig. 3) at equivalent temperatures.

Zacheo et al. (2000) reported that LOX in almonds remained unchanged after a 10 min exposure to 40 °C, while LOX was inactivated as temperature increased. LOX in almonds lost 100% activity after a 10 min exposure to 80 °C. Zacheo et al. (2000) also reported that LOX activity in almonds depends upon cultivars. Cultivar Padula di Ruvo exhibited greater LOX activity than cvs. Sannicandro, Fra Giulio Grande, or Desmayo Largueta.

Buranasompob (2001) demonstrated no significant differences ($P > 0.05$) in peroxide values between oils extracted from untreated almond kernels and oils extracted from short-time heat-treated almond kernels after 5, 15, or 30 d of storage at 25 °C, or after 20 d of storage at 35 °C. Zacheo et al. (2000) reported that LOX activity in almond kernels remain unaltered or increased with aging time, and that in-shell almonds did not exhibit significant changes in peroxide values after 2 years of storage at room temperature.

3.4. Homogenates of walnut kernels

Table 1 presents percentage inhibition of soybean LOX activity by homogenates of untreated and short-time heat-treated walnut kernels. Activities of the control soybean LOX solution and homogenates of walnut kernels were determined to assess antioxidant activity of shelled walnut kernels. The initial mean LOX activity of the blank soybean LOX solution was 1.58 μM O_2/l s. The initial mean LOX activity of the homogenates of untreated walnut kernels was 0.26 μM O_2/l s. The initial mean LOX activity of the homogenates of short-time heat-treated walnut kernels heated at 55 °C for 2 or 10 min, or 60 °C for 2 or 10 min were 0.12, 0.10, 0.09, and 0.05 μM O_2/l s, respectively.

The mean LOX activity of the homogenates of untreated walnut kernels plus soybean LOX was 0.66 μM O_2/l s. The mean LOX activity of the homogenates of short-time heat-treated walnut kernels heated at 55 °C for 2 or 10 min, or at 60 °C for 2 or 10 min plus soybean LOX were 0.60, 0.60, 0.55, and 0.55 μM O_2/l s, respectively. The control soybean LOX solutions exhibited greater LOX activity than the homogenates of both untreated and short-time heat-treated walnut kernels (Table 1). Walnut kernels may contain natural antioxidants, heat stabile putative antioxidants, or LOX inhibitors resulting in determination of decreased LOX activity. In this study, preparation of the homogenates of walnut kernels were not purified. Therefore, specific antioxidants or enzyme inhibitors were not identified.

3.5. Homogenates of almond kernels

Table 1 presents inhibition of soybean LOX activity by homogenates of untreated and short-time heat-treated almond kernels. The initial mean LOX activity of the blank soybean LOX solution was 1.58 μM O_2/l s. The initial mean LOX activity of the homogenates of untreated almond kernels was 0.11 μM O_2/l s. The initial mean LOX activity of the homogenates of short-time heat-treated almond kernels heated at 55 °C for 2 or 10 min, or 60 °C for 2 or 10 min were 0.09, 0.03, 0.09, and 0.03 μM O_2/l s, respectively.

The mean LOX activity of the homogenates of untreated almond kernels plus soybean LOX was 0.47 μM O_2/l s. The mean LOX activity of the homogenates of short-time heat-treated walnut kernels heated at 55 °C for 2 or 10 min, or at 60 °C for 2 or 10 min plus soybean LOX were 0.39, 0.21, 0.29, and 0.17 μM O_2/l s, respectively. The control soybean LOX solution exhibited greater LOX activity than the homogenates of both untreated and short-time heat-treated almond kernels (Table 1). Almond kernels may contain natural antioxidants or enzyme inhibitors which inhibited LOX activity in the homogenates of almond kernels.

Table 1
Lipoxygenase and antioxidant activities of short-time heat-treated walnut and almond homogenates

	Activity (μM O_2/l s)				
	Control	55 °C 2 min	55 °C 10 min	60 °C 2 min	60 °C 10 min
Walnuts (W)	0.26[c]	0.12[d]	0.10[d,e]	0.09[d,e]	0.05[e]
Almonds (A)	0.11[f]	0.09[f]	0.03[g]	0.09[f]	0.03[g]
Mix (W + S)	0.66[a]	0.60[b]	0.60[a,b]	0.55[b]	0.55[b]
Mix (A + S)	0.47[a]	0.39[b]	0.21[d]	0.29[c]	0.17[e]
% Inhibition					
Walnuts	64%	65%	64%	67%	66%
Almonds	72%	77%	87%	83%	90%

Control soybean LOX activity was 1.58 μM O_2/l s.
The weight ratio of walnut or almond homogenate to soybean LOX was 4:1.
*Values within columns and rows followed by different superscripts are significantly different ($P \leqslant 0.05$).

3.6. Inhibition in LOX activity in the homogenates of walnut and almond kernels

The homogenates of untreated walnut kernels exhibited greater LOX activities than the homogenates of untreated almond kernels (Table 1). Walnut kernels contain greater concentrations of linoleic and linolenic acids than almond kernels (Macrae et al. 1993). Greater concentrations of linoleic and linolenic acids lead to susceptibility to oxidative rancidity, which produces undesirable volatile compounds and off-flavors.

Table 1 presents percentage inhibition of soybean LOX activity by homogenates of walnut and almond kernels. The homogenates of walnut kernels exhibited smaller inhibition of LOX activity than the homogenates of almond kernels.

The USDA (1983) reported that walnut kernels contain smaller concentrations of α-tocopherol (~2.62 mg/100 g) than almond kernels (~24.01 mg/100 g). Zacheo et al. (2000) reported that α-tocopherol in almonds retard the lipid oxidation and prolong the shelf-life of almonds. Macrae et al. (1993) and Young and Cunningham (1991) stated that almonds and almond products exhibit a longer shelf-life compared to other nuts because almonds and walnuts contain smaller concentrations of polyunsaturated fatty acids and larger concentrations of antioxidant α-tocopherol. Zacheo et al. (2000) also reported that α-tocopherol in almond kernels is an important antioxidant protecting almonds against lipid oxidation leading to prolonged shelf-life. α-Tocopherol is a natural antioxidant and may inhibit LOX and related oxidative rancidity. Zacheo et al. (2000) reported that LOX activity of almond kernels increases with storage time, recommending continued investigation to assess the association of LOX activity, antioxidant concentration, and lipid oxidation with oxidative rancidity of almonds.

4. Conclusions

Short-time heat treatments of 55 or 60 °C for 2 or 10 min inactivate LOX enzymes in walnut and almond kernels. The homogenates of untreated walnut kernels exhibited greater LOX activity than the homogenates of untreated almond kernels. Upon the addition of the homogenates of walnut or almond kernels to soybean LOX to assess antioxidant activity, the homogenates of walnut kernels exhibited less inhibition of LOX activity than the homogenates of almond kernels. Short-time heat treatments 55 °C for 2 min or greater reduce LOX activity and retard the development of oxidative rancidity in shelled walnuts and almonds during distribution and storage, and extend the shelf-lives of walnuts and almonds.

Acknowledgements

This research was presented as a poster (Abstract No. 14B-17) at the 2000 Institute of Food Technologists Annual Meeting, Dallas, Texas., USA. Financial support from USDA (NRI Grant No. 962-35316 and IFAFS 00-52103-9656), International Marketing Program for Agricultural Commodities and Trade (IMPACT) at Washington State University, and Boonrawd Brewery Co., Ltd. (Bangkok, Thailand) is gratefully acknowledged.

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