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Lipoxygenase activity in walnuts and almonds

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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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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