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Investigation of drying conditions on bioactive compounds, lipid oxidation, and enzyme activity of Oregon hazelnuts (*Corylus avellana* L.)

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ABSTRACT

Hazelnut kernels obtained from the commercial processes were analyzed for proximate composition, bioactive compounds, lipid oxidation, and enzyme (lipase, peroxidase, and polyphenol oxidase) activities and compared among three cultivars (Barcelona, Yamhill, and Jefferson) and two agricultural practices (with and without fertigation for Jefferson). The influence of drying temperature (38, 43, and 49 °C) and relative humidity (RH, 40 and 60%) on drying characteristics (drying rates of inshells and corresponding moisture content (MC) and water activity (a_w) of kernels when inshells reached ~10 g/100 g MC or equilibrium MC (MC_{eq})), bioactive compounds, lipid oxidation, and enzyme activity of the kernels were investigated. Oleic acid and α-tocopherol were the predominant fatty acids and vitamin in hazelnuts. Yamhill contained the highest phenolic (0.12 mg GAE/g), vitamin E (14.26 mg/100 g, α-tocopherol), and lowest unsaturated/saturated fatty acids ratio (8.7) among cultivars. Drying characteristics and chemical and enzymatic reactions of hazelnuts varied depending on drying condition and cultivar of nuts, in which drying at 43–49 °C and 40% RH improved drying efficiency and retained quality of dried hazelnuts. This study suggested the ideal drying conditions that could produce dried hazelnuts with optimal range of MC and a_w for retaining bioactive compounds and minimizing lipid oxidation and enzyme activity.

1. Introduction

The United States (US) is the third largest hazelnut (*Corylus avellana* L.) producing country and the export value was about $117.7 million in 2014–2015 with a steady increase over the past five years (Romero, 2015). The state of Oregon produces 99% of the total USA hazelnut crop, and various cultivars are available, including Barcelona, Yamhill, and Jefferson (Olsen, Mehlenbacher, McCluskey, & Smith, 2013). Yamhill and Jefferson are advanced cultivars with reduced kernel defects and increased resistance to fungal growth (McCluskey, Mehlenbacher, & Smith, 2009; McCluskey, Mehlenbacher, & Smith, 2011). Fertigation (addition of fertilizers into the water irrigation system) is applied to hazelnut trees for enhancing the absorption of the recommended nutrients and fertilizers (Canali, Nardi, Neri, & Gentili, 2005). Hazelnuts contain a good quantity and quality of fat and protein along with high content of vitamin E and phenolic compounds, while the exact amount depends on cultivars, fertigation treatment, or growth region (Köksal, Artik, Şimşek, & Güneş, 2006; Oliveira et al., 2008). Unfortunately, there has been no report, to our knowledge, on chemical compositions, bioactive compounds, lipid oxidation, and enzyme activity of different cultivars harvested from Oregon to differ them with respect to their quality characteristics.

Drying is essential while processing postharvest hazelnut inshells (unshelled hazelnuts) for ensuring food safety and quality during storage. The recommended safe moisture content (MC) for inshells and kernels is ~10 g/100 g and ~6 g/100 g, respectively (Gross, Wang, & Saltveit, 2014), which has been used as the industrial standard. The high content of unsaturated fatty acids in hazelnut kernels can be susceptible to lipid oxidation during the drying process due to exposure to oxygen and high temperature (López et al., 1997a). In addition, undesirable range of MC and water activity (a_w) of kernels as a result of drying can increase hydrolytic or oxidative enzyme activities, including lipase (LP), peroxidase (POD), and polyphenol oxidase (PPO). Hence, drying process should be applied carefully to postharvest hazelnut inshells for not only suppressing microbial growth, but also delaying quality deterioration associated with lipid oxidation and enzymatic reaction in kernels.

Both temperature and humidity had been considered as the critical factors for not only improving the drying efficiency, but also retaining

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the quality of dried products (Kashaninejad, Mortazavi, Safekordi, & Tabil, 2007; Palipane & Driscoll, 1994; Omid, Baharlooei, & Ahmadi, 2009; Pankaew, Janjai, Nilnont, Phusampao, & Bala, 2016). In the commercial hazelnut processing facility, freshly harvested hazelnuts are commonly dried using hot-air tunnel dryer under a temperature of 100 °F (~38 °C) for ~24 h. For improving the drying efficiency and minimizing lipid oxidation of dried hazelnuts, higher temperatures (~43 °C (110 °F) and ~49 °C (120 °F) with 10 °F interval), but lower than 50 °C, were investigated and compared with ~38 °C (100 °F) which was commercially used in the industry. According to López et al. (1997a), lipid oxidation of hazelnuts could be accelerated while drying at temperature higher than 50 °C. For relative humidity (RH), two levels (40 and 60%) with broader interval were selected for firstly investigating the influence of RH on drying characteristics of hazelnut inshells. This study thus evaluated the influences of six different drying conditions combined with temperature (38, 43, and 49 °C) and RH (40 and 60%) on drying characteristics, bioactive compounds, lipid oxidation, and enzyme activity of different hazelnut cultivars produced in Oregon.

The specific objectives of this study were 1) to quantify the important bioactive compounds, lipid oxidation products, and enzyme activity of commercially processed Oregon hazelnut kernels (Barcelona, Yamhill, Jefferson, and Jefferson with fertigation (Jef-fertigation)) and 2) to investigate the impact of six combinations of forced-air drying conditions on the drying characteristics (drying rates of inshells and corresponding MC and a_w of kernels when inshells reached to ~10 g/100 g MC or equilibrium MC (MC_{eq})), bioactive compounds (vitamin E (VE), total phenolic content (TPC), DPPH free radical scavenging activity (DPPH)), lipid oxidation (primary and secondary lipid oxidation products, and acid value), and enzyme activity (LP, POD, and PPO) of hazelnut kernels. This study will provide scientifically based guideline to hazelnut industry for drying nuts with minimal loss in bioactive compounds and important quality parameters while ensuring food safety for each of the predominant Oregon hazelnut cultivar.

2. Materials and methods

2.1. Materials

Fresh Oregon hazelnut inshells (Barcelona, Yamhill, Jefferson, and Jefferson with fertigation (Jef-fertigation)) and commercially processed inshells and kernels were provided by the Oregon Hazelnut Marketing Board during September to October 2015. Commercially processed inshells (washing and drying) and kernels (washing, drying, cracking, and sorting) were kept in a dark room at −18 °C prior to chemical analysis. Fresh inshells were stored at 1 °C until the whole drying process finished.

Chemicals were obtained from different manufacturers: methanol from EMD Millipore (Billerica, MA, USA); Folin-Ciocalteu (FC) reagent, gallic acid, hexane, formaldehyde, diethyl ether, sodium phosphate monobasic, sodium phosphate dibasic, phenolphthalein, sodium sulfate, catechol, zinc acetate, potassium ferrocyanide and *o*-dianisidine from Sigma-Aldrich (St. Louis, MO, USA); hydrochloride, chloroform, potassium hydroxide from Fisher Scientific (Hampton, NH, USA); 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (950 g/kg) from Alfa Aesar (Ward Hill, MA, USA); hydrogen peroxide, sodium hydroxide, calcium chloride, acetic acid and L-ascorbic acid from MACRON, Avantor Performance Materials (Center Valley, PA, USA); sodium carbonate and polyvinylpyrrolidone (PVPP) from VWR International (Radnor, PA, USA). Carrez solution I was prepared by dissolving 21.9 g of zinc acetate and 3 g of glacial acetic acid in water to make up to 100 mL, and Carrez solution II was prepared by dissolving 10.6 g of potassium ferrocyanide in water to make up to 100 mL.

2.2. Proximate composition

Commercially-processed kernels were utilized for measuring proximate composition. Moisture content (MC) was measured using a non-destructive method by the Steinlite Moisture Tester (SB 900, Seedburo Equipment Co., Des Plaines, IL, USA). Fat, protein, and ash contents were analyzed following AOAC standard methods (AOAC, 2000). They are Soxhlet extraction method for total fat (method 920.39C), Kjeldahl method for protein (method 992.15), and ash content by direct ignition in a furnace at 525 °C for 5 h (method 942.05). The percentage of crude protein was estimated by multiplying the total nitrogen content with a factor of 6.25. Carbohydrate content was calculated by subtracting contents of other compositions from 100%. Data were reported as a percentage of kernels.

2.3. Fatty acid profiles

Fatty acid profiles of commercially processed kernels were measured by a gas chromatograph (GC) using the method of Aziza, Panda, Quezada, and Cherian (2013). About 2 g of powdered kernels were extracted with 9 mL of chloroform: methanol (2/1 mL/mL), and the lipid extract was treated with 3 mol/L methanolic hydrochloride to form fatty acid methyl esters. The fatty acid distribution was identified using an Agilent 6890 GC (Model 6890, Agilent, Santa Clara, CA, USA) equipped with an auto-sampler, flame-ionization detector (FID), and fused-silica capillary column (30 m × 0.25 mm × 0.2 μm film thickness). One μL of each sample was injected with helium as a carrier gas onto the column programmed for increased oven temperatures (the initial temperature of 110 °C was held for 0.5 min, increased at 20 °C/min to 190 °C, held for 7 min, and then increased at 5 °C/min to 210 °C and held for 8 min). Temperature of inlet and detector was set to 250 °C. Fatty acid methyl esters were identified by comparison with retention times of authentic standards, and quantified using the Agilent ChemStation software. Results were expressed as percentages of fatty acid methyl esters on lipid extracts from kernels.

2.4. Bioactive compounds

VE, TPC, and DPPH were determined for hazelnut kernels. VE content (both α- and γ-tocopherols) was quantified by high performance liquid chromatography (HPLC) with electrochemical detection (ECD) (Podda, Weber, Traber, & Packer, 1996). Briefly, 1 g of hazelnut kernel powder was saponified with ethanol-KOH solution consisting of 10 mL ethanol and 1.5 mL potassium hydroxide (12.1 g/L), extracted with hexane, dried under nitrogen, and then re-suspended in ethanol-methanol solution (1/1 mL/mL). The HPLC system operated consisted of a controller (Shimadzu, LC-10ADvp, Kyoto, Japan), an auto-injector (Shimadzu, SIL-10ADvp, Kyoto, Japan) with a 50 μL sample loop, and amperometric ECD detector (LC-4B ECD, Bioanalytical Systems Inc., West Lafayette, IN, USA) with a glassy carbon working electrode and a silver chloride reference electrode. The column used was a Synergi Hydro-RP, 4 μm, 150 × 4.6 mm (Phenomonex, Torrence, CA, USA) with a Security Guard Cartridge, 4.0 × 3.0 mm (Phenomonex, Torrence, CA, USA). The mobile phase was made by 990 mL/L methanol containing 100 g/L lithium perchlorate. Both tocopherols were identified using authentic standards (α- and γ-tocopherols from Henkel (La Grange, IL, USA), and the peak areas were integrated using Shimadzu Scientific 4.2 Class VP software package. Samples were analyzed in triplicate and presented as mg/100 g kernels.

TPC analysis was conducted on the methanolic extracts of defatted hazelnut kernels and determined by Folin-Ciocalteu assay (Singleton & Rossi, 1965). Briefly, 0.5 mL of extracts or gallic acid (0.1, 0.3, 0.5, 0.7, and 1.0 mg/mL) were mixed with 0.5 mL of FC reagent and 7.5 mL of distilled water, respectively. The mixtures were vortexed and rested for 20 min in the ambient temperature. After adding 3 mL of 200 g/L sodium carbonate, the mixture was further incubated at 40 °C water bath for 20 min. The reaction mixture was cooled down in ice bath for 3 min. The absorbance was read at 765 nm using a UV/Vis spectrophotometer (UV160U, Shimadzu Corporation, Kyoto, Japan). Distilled water was used as a blank. Results (n = 3) were expressed as gallic acid equivalent (GAE) mg/g kernels.