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Active edible coating from chitosan incorporating green tea extract as an antioxidant and antifungal on fresh walnut kernel

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ABSTRACT

The effects of coatings made from chitosan (Chi) incorporating green tea extract (GTE) on lipid oxidation, fungal growth, and sensory properties of walnut kernels were studied. The Chi powder and GTE obtained from green tea were combined to obtain the final concentrations of Chi-GTE: 5-0, 5-5, 5-10, 10-0, 10-5 and 10-10 g L^{-1} in an aqueous coating solution. Effective inhibition of lipid oxidation and fungal growth during storage of walnut kernels (18 weeks) was possible using the Chi10 coating combined with GTE. Different proportions of GTE had no significant effect ($p < 0.05$) on lipid oxidation. Fungal growth was not detected during the storage period with Chi10 and all different proportions of GTE, Throughout the storage duration, coatings without GTE showed no significant ($p < 0.05$) effect on sensory properties; however Chi10-GTE10, was significantly ($p < 0.05$) unacceptable by panelist. The results suggested that Chi10-GTE5 could prolong the shelf life of walnut kernels.

1. Introduction

Unsaturated fatty acids in nuts and unfavorable environmental conditions make them vulnerable to lipid oxidation (Bonilla et al., 2012) and fungal growth (Maghsoudlou et al., 2012). Previous studies were performed to understand treatments that affect quality maintenance of nuts such as the effect of packaging and storage conditions on quality of raw shelled walnuts (Mexis et al., 2009 and Bakkalbas et al., 2012), and almond (Raisi et al., 2015). Coating is a specific form of films directly applied to the surface of materials and regarded as a part of the final product (Han and Gennadios, 2005). Edible active coatings are biodegradable and increase food protection (Geraldine et al., 2008) because of functional properties such as antioxidant agents, antimicrobial agents or both, and act as an oxygen barrier that can limit oil oxidation (Atarés et al., 2011) and fungal growth (Sayanjali et al., 2011). Chitin is a polysaccharide composed from *N*-acetyl-D-glucosamine units. Chitin is found in invertebrates, insects, marine diatoms, algae and fungi and is ordinarily converted to chitosan by deacetylation in concentrated alkali solution (Maghsoudlou et al., 2012). Chitosan is a safe, natural, allergen-free and biocompatible polymer with health benefits (Bornet and Teissedre, 2011). Chitosan coatings have been successfully used in the food industry, mostly because of their structural properties (Devlieghere et al., 2004), that allow formation of a continuous layer of coating on foods. Antimicrobial properties (Dutta et al., 2009; Liu et al., 2006; Maghsoudlou et al., 2012; Sebti et al., 2005) and antioxidant activity (Kamil et al., 2002; Xing et al., 2005; Xue et al., 1998) of chitosan are interesting for the food industry. Superoxide radical scavenging activity of free hydroxyl and amino groups of chitosan can inhibit disintegration of superoxide radicals and development of robust reactive oxidative species such as singlet oxygen and hydroxyl radicals, which initiate peroxidation of lipids (Xing et al., 2005; Xue et al., 1998). Different mechanisms have been proposed for the antimicrobial activity of chitosan: (1) interaction between positively charged chitosan molecules and negatively charged microbial cell membranes leads to the leakage of intracellular constituents (Helander et al., 2001; Liu et al., 2004); (2) chelation of trace metals and inhibition of the production of toxins and microbial growth; and, (3) binding with DNA and inhibiting mRNA synthesis (Rabea et al., 2003). Some limitations of chitosan as an applied antioxidant and antifungal exist, such as identifying the optimum concentration of chitosan coating solution in order to retard oxidation and fungal growth without undesirable effects on sensory properties of the final product (Devlieghere et al., 2004; Maghsoudlou et al., 2012). These limitations can be mitigated with green tea added as an antioxidant

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(Gramza et al., 2006; Lee et al., 2007; Rohn et al., 2004; Siripatrawan and Harte, 2010) and antimicrobial agent (Maghsoudlou et al., 2012; Rohn et al., 2004; Siripatrawan and Noipha, 2012; Yam et al., 1997). Most antioxidant activity of green tea is related to the polyphenolic compounds in tea known as catechins (Lee et al., 2007), with epicatechin gallate, epicatechin and catechin having the highest antioxidant activity (Gramza et al., 2006). Polyphenolic compound of green tea extract act as an antioxidant through prevention of radical chain initiation, binding of transition metal ion catalysts, and interaction with the free radicals to inhibit lipid oxidation (Farhoosh et al., 2007; Perumalla and Hettiarachchy, 2011; Siripatrawan and Noipha, 2012). Antimicrobial activity of green tea extract is related the content of catechins (Yam et al., 1997). Hydroxyl moieties at 3′, 4′ and 5′ on the B ring in the catechin molecules are major factors of antimicrobial activity of green tea (Koech et al., 2013). Green tea extract also can reinforce the mechanical properties of coatings (Siripatrawan and Harte, 2010), and thus the network of the coating can act as better barrier to oxygen and limit lipid oxidation and fungal growth. Previous research studies have proved that the application of chitosan coating incorporating natural antioxidant and antimicrobial agents improves the storability of several perishable foods, including incorporation of olive oleoresin to improve the antioxidant protection processed butternut squash (Ponce et al., 2008), fish oil reduced lipid oxidation and microbial growth in Ling cod fillets (Duan et al., 2010), *Origanum vulgare* L. essential oil controlled postharvest pathogenic fungi in grapes (dos Santos et al., 2012), lemon essential oil enhanced antifungal activity in strawberries (Perdones et al., 2012) and essential oils to control the microorganisms present in fresh cut broccoli (Alvarez et al., 2013). The aim of this study was to evaluate the effect of chitosan incorporating green tea extract as an edible coating on antioxidant activity, antifungal activity and sensory properties of walnut kernels.

2. Materials and methods

2.1. Materials

Walnut fruit (*Juglans regia* L., Kaghazi cultivar) were obtained in the summer from Baft, Kerman, Iran. Walnut fruit were hand-picked directly from the trees and immediately carried to the laboratory at the University of Agricultural Sciences and Natural Resources, Gorgan. Iran. Walnut fruit, with the green skin manually removed, were dried in an oven at 30 ◦C for 1 d. Then, their hard shell was manually removed. Green tea (*Camellia sinensis*, var. sinensis) leaves were picked in the summer from Roodsar, Gillan, Iran, and immediately carried to laboratory. All materials used in this study for chemical and microbial analysis were purchased from Merck (Germany) and Sigma–Aldrich (USA).

2.2. Methods

2.2.1. Extraction and preparation of coatings

According to the method of Maghsoudlou et al. (2012) Chi (crab shell chitosan, with degree of deacetylation ≥0.75) solutions at concentrations of 5 and 10 g/L in aqueous coating solutions, were prepared by dissolving chitosan powder in an aqueous solution of glacial acetic acid (1 mL in 100 mL), under stirred conditions at 60 ◦C and then glycerol at the ratio of 1–4 chitosan powder was added to the Chi solution as a plasticizer. Stirring of the Chi solution was continued for 15 min. According to the method of Lin et al. (2008), green tea leaves were subjected to blanching (95–100 °C, 40–45 s), rolling and drying at room temperature. The dried green tea leaves were ground using a blender (Sunny SFP-820), and screened through a mesh (0.231 mm sieve size). Total phenolic content of green tea was determined with Folin–Ciocalteu reagent using gallic acid as a standard according to the method described by Claudia et al. (2008). The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10 to 50 mg/L (Pearson's correlation coefficient: $r^2 = 0.9996$). The content of total phenols, expressed as gallic acid, was 761.5 ± 42 g/kg of dried matter. According to the method of Siripatrawan and Harte (2010) with slight modification, green tea powder was kept in a linear low density polyethylene laminated aluminum pouch under vacuum conditions using a vacuum packaging sealer (Henkelman B.V., P.O. Box 2117) and was stored in at 4 °C until extraction. GTE solution was prepared by mixing ground green tea powder in distilled water (1:5 w-w) and was controlled at 80 °C in an Erlenmeyer flask and stirred in a water bath shaking incubator (Memmert D-91126) for 15 min, to achieve maximum phenolic compound extraction. The GTE was filtered through Whatman No. 1 (11 μm) filter paper and was added tp the Chi solution. The solutions was cooled to room temperature.

2.2.2. Storage stability studies

Chi and GTE were blended to obtain the final concentrations of Chi-GTE: 5-0, 5-5, 5-10, 10-0, 10-5 and 10-10 g/L in aqueous coating solutions. The resulting solutions containing GTE were homogenized using a homogenizer (Heidolph, D-91126, Schwabach-Germany). Non coated treatment was used as the control. After weighing, walnut kernels were placed in a mesh container and immersed in the appropriate coating solution for 1 min. Coated walnut kernels were then placed on a tray and dried in an oven at 35 °C for 5 h. Then, coated samples and control samples were packed in 50 g polyethylene bags, sealed with packaging sealer, and kept at room temperature for 18 weeks. During storage, samples were examined tri-weekly for the following analyses.

2.2.2.1. Peroxide value (POV). Separation of lipids was conducted by the method of Kang et al. (2013). Walnut kernels (25 g) were smashed using a mortar, after which n-hexane at a volume double that of the smashed walnuts was added. Lipids were extracted in a shaking incubator at 30 °C for 12 h, after which lipids containing hexane were processed by centrifuge at 10.308 × *g* for 15 min. The fine walnut powder and pieces were completely separated using a Whatman No. 1 (11 μm) filter paper. The hexane in the separated supernatant was removed using a rotary evaporator (IKA, Germany) at 50 °C. POV was evaluated using a acetic acid–chloroform method. POV was reported as mass of oxygen per mass of oil, g/kg (Shahidi and Zhong, 2005).

2.2.2.2. Thiobarbituric acid (TBA) value. Secondary oxidation products of oils and fats, especially malondialdehyde, react with 2-thiobarbituric acid and form pink complexes. Spectrophotometric quantitation of the pink complex is measured at 530 nm. The thiobarbituric acid reactive substance (TBARS) changes was reported as mass of malondialdehyde per mass of oil, g/kg (Shahidi and Zhong, 2005).

2.2.2.3. Microbial analysis. Fungal activity was determined according to the method of Devlieghere et al. (2004) with slight modification. Ten grams of walnut kernels were smashed and homogenized in 90 mL of sterile NaCl solution (8.5 g/L) for 2 min using a Stomacher Lab Blender (Seward Laboratory, London, UK). For yeasts and molds, the samples were spread-plated on YGC media and incubated at 25 °C for 5 d. The growth yeast and mold was reported as log colony forming units (CFU) per kilogram.

2.2.2.4. Sensory evaluations. Sensory evaluation was also performed according to the method of Maghsoudlou et al. (2012) with a 5 point Hedonic scale, (1 = bad, 2 = poor, 3 = mean, 4 = good and 5 = very good). Each panelist was given four walnut