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Glycerol ginger extract addition to edible coating formulation for preventing oxidation and fungal spoilage of stored walnuts

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ABSTRACT

During storage, walnuts can undergo oxidation processes that lead to a decay of product quality and the development of rancidity and off-flavor. The present study investigated how the application of a chitosan coating, containing a glycerol ginger extract (GGE) rich in bioactive compounds, can preserve walnut quality during storage. Glycerol can be considered a green extraction solvent, which allows to obtain extract rich in polyphenols and with high radical scavenging activity. GGE employed in the formulation of a chitosan-based coating determined significantly lower ($p < 0.05$) lipid oxidation and peroxides values in walnuts stored under stressing temperature conditions (45 °C), in comparison to Control (uncoated sample) and even in comparison to the walnuts coated with sole chitosan. Moreover, when artificially inoculated with *Aspergillus flavus* spore suspension, walnuts treated with GGE-enriched coating evidenced the lowest disease incidence and the lowest spore concentration. Results obtained encourage the application of coating containing GGE even in other rich lipid matrices that can easily undergo oxidation process or spoilage caused by phytopathogenic fungi.

1. Introduction

Among nuts, walnuts (*Juglans regia* L.) evidence high content in polyunsaturated fatty acids (Bamberger et al., 2017) and many research studies confirm how their consumption can be associated with various beneficial effects against chronic diseases (Bakkalbaşi et al., 2012; Yi et al., 2022). Moreover, due to its optimal content in ω-3 and ω-6 polyunsaturated fatty acids, walnut oil has been broadly studied as ingredient for improving the nutritional characteristics of a wide range of food products (Ayo et al., 2007). Unfortunately, transportation and handling, carried out at unsuitable conditions, can negatively affect the kernel quality inducing browning and oxidation. In particular, the temperature has been designated as the most crucial factor among others, like oxygen and light, for quality deterioration of walnut kernels (Mexis et al., 2009). In fact, walnut lipids are highly susceptible to oxidation which leads to the rancidity of the products (Shahidi & John, 2013) and compromises the overall nutritional characteristics, as well as sensory and texture parameters (Yi et al., 2014). The growing attention towards the consumption of healthy foods has led many consumers to choose nuts as an alternative snack (Alasalvar et al., 2020). However, the nut market is threatened by potential food safety and quality concerns arising from potential contamination by aflatoxigenic molds, such as *Aspergillus flavus*, able to grow at several environmental factors and to produce aflatoxin B1 (AFB1), which is considered the most recurrent and also the most harmful among aflatoxins (Mirabile et al., 2021; Ostry et al., 2017; Parafati et al., 2022). All the mentioned changes can induce a reduction in economic value of the product, thus it is very important to preserve the product quality along the entire supply chain. In order to safeguard the quality of walnuts during storage, different authors have proposed edible coating and natural plant substances as a new strategy to prevent oxidation (Chatrabnous et al., 2018; Grosso et al., 2020; Habibi, Yazdani, Chatrabnous, Koushesh Saba, & Vahdati, 2022; Habibie et al., 2019; Sarikhani et al., 2021). Besides, as widely demonstrated in previous research studies, vegetable extracts, having substantial antioxidant potential, can be incorporated into the coating in order to improve the storability of several perishable foods such as cheese, fish fillets, or to prevent the decay of fruits during post-harvest period (Alsaggaf et al., 2017; Kharchoufi et al., 2018; Molina-Hernández et al., 2020).

Among vegetable matrix, ginger (*Zingiber officinale*) is a plant rich in phytochemicals and already investigated for its beneficial effects against obesity, diabetes, cardiovascular and chronic kidney disease. The use of

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different extraction methods has also allowed different researchers to obtain extracts with good content of total polyphenols and high antioxidant activity, despite some of the extracts investigated could not be used in food formulations due the toxicity of the extraction solvent (Ezez & Tefera, 2021). In addition to its antioxidant activity, different authors evidenced as ginger extract or essential oil can slow down the oxidation of meat (Barkhordari & Bazargani-Gilani, 2021) or fish products (Cai & Wang, 2021) acting on protein stability, but little or nothing is reported about its ability to inhibit the lipid oxidation of nuts.

To the best of our knowledge, only the study of Sabaghi et al. (2015) proposed the use of a coating incorporating a vegetable extract to prevent lipid oxidation in stored walnuts.

Therefore, the aims of the present study were: i) to maximize the bioactive compounds' extraction through the use of a green solvent, such as glycerol; and ii) to incorporate the obtained glycerol ginger extract (GGE) into a chitosan coating, in order to enhance the oxidative stability of walnut kernels stored under stressing temperature condition. Moreover, the final goal of the research was to estimate the anti-fungal properties of the aforementioned enriched coating against *A. flavus*, artificially inoculated in walnuts.

2. Materials and methods

2.1. Sample preparation

Ginger root sample, imported from China (as indicated on the commercial label), was bought from a supermarket in Catania (Sicily, Italy) and immediately transported to the Food technology laboratory at the Department of Agriculture, Food and Environment (Di3A), University of Catania.

Ginger root was hand-peeled with a knife and then dried at 42 ± 1 °C for 48 h. After drying treatment, ginger was grinded and the obtained flour was ground down using 38 mesh sieves (0.5 mm) to standardize the particle size. The obtained ginger flour was evaluated for moisture content using a thermobalance (Eurotherm, Gibertini®, Novate Milanese, Italy), vacuum packed and stored at −20 ± 1 °C for the subsequent analysis.

2.2. Extraction of bioactive compounds

Ginger sample, prepared as described before, was subjected to extraction of bioactive compounds using glycerol as solvent. In brief 10 g of ginger powder was homogenized in 100 mL of glycerol and let to stir, in the dark, for 24 h at 50 ± 2 °C (extraction procedure was previously optimized, data not shown). After that, glycerol ginger suspension was centrifuged at 10.000×*g* (ALC 4239R, Winchester, VA, USA) for 10 min and vacuum filtered through filter paper. The obtained glycerol ginger extract (GGE) was evaluated for its content in total polyphenols, total flavonoids and antioxidant activity as reported below (paragraph 2.2.1.)

2.2.1. Evaluation of total polyphenols, total flavonoids and antioxidant activity of glycerol ginger extract

GGE was evaluated for its content in total polyphenols through the Folin-Ciocalteau assay described by Vazquez-Roncero et al. (1973) with slight modifications.

In brief, a volume equal to 250 μL of the extract was mixed with 12.5 mL of distilled water and 1.25 mL of Folin-Ciocalteau (FC) reagent (Carlo Erba Reagents, Italy). After 3 min, 2.5 mL of sodium carbonate (20%) were added and all the mixture was brought to a final volume of 25 mL with distilled water. The graduated flask was then placed under dark conditions for 1 h at room temperature (20 °C). The corresponding blank was prepared, using pure glycerol instead of extract. Thus, the absorbance of the extract was spectrophotometrically measured against the blank solution at 725 nm by utilizing a PerkinElmer lambda 25 UV–Vis spectrometer (PerkinElmer, Inc., MA, USA). The results of total phenolic content were expressed as mg/mL of GGE of gallic acid equivalents (GAE) attended against the standard curve of gallic acid (mg GAE/mL).

The amount of total flavonoids of GGE under study was evaluated using the aluminum chloride colorimetric assay, reported by Lin and Tang (2007), with minor modifications. An amount of 0.5 mL of GGE was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$), 0.1 mL of 1 M potassium acetate (CH_3COOK) and 2.8 mL of deionized water. Blank was prepared by replacing the GGE with pure glycerol. Sample was let to stand in the dark at room temperature (20 °C) for 40 min, after which the absorbance was spectrophotometrically measured at 415 nm against the blank. A seven-point standard curve was constructed using quercetin 3-β-d-glucoside as a standard and results were expressed as quercetin equivalents (mg QE/mL).

GGE was also evaluated for its Radical Scavenging Activity (RSA) through the DPPH assay. The method followed was that of Brand-Williams et al. (1995) which was carried out by combining 50 μL of ginger extract with 3 mL of methanol DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (100 M). The mixture was homogenized and then incubated for 1 h at room temperature (20 °C) in the dark. The same steps were used to prepare the blank sample, but the amount of extract was swapped out for methanol. The absorbance of each sample was measured at 515 nm at the end of the reaction time using a Perkin Elmer Lambda 25 Uv–Vis spectrometer.

RSA of GGE was expressed as percentage and calculated following the formula:

$$RSA\% = \left(\frac{A_b - A_s}{A_b}\right) \text{ x } 100$$

Where: A_b is the absorbance of the blank and A_s is the absorbance of the sample.

All the analysis above mentioned were performed in triplicate.

2.3. Preparation of edible coating carrying glycerol ginger extract (GGE)

Usually glycerol is added to the coating formulations as a plasticizer; therefore, to formulate the chitosan coating, GGE was integrated for its dual functionality, as a plasticizer and as a source of bioactive compounds. The GGE coating was prepared following the method reported by Grilo et al. (2020), with minor modification as follows: 1.5 g of chitosan (deacetylation degree >75%, viscosity ≤200 mPa s in 1% acetic acid, molecular weight ~ 150,000 Da, Sigma Aldrich, Steinheim, Germany) was dissolved into 75 mL of 1% glacial acetic acid solution (Carlo Erba Reagents, Italy) using a magnetic stirrer set at 50 °C. Then, 25 mL of GGE was added to the chitosan-acidified solution and stirred again until the uniformity of the coating solution was reached. Control coating was made using pure glycerol instead of GGE.

2.4. Walnut treatment with edible coating containing glycerol ginger extract (GGE)

The whole walnuts were purchased from a supermarket located in Catania (Sicily, Italy). Fruits, from California-USA (as indicated on the commercial label), have been shelled manually, through a nut-cracker, to collect the fresh walnut kernels. After shelling, a walnut portion was immediately analyzed (as reported in the following paragraphs) in order to establish the initial characteristics of the product. Subsequently, once a suitable quantity of kernels has been obtained (about 1 Kg for each of the 3 independent replicates), walnut kernels were divided into three experimental lots and subjected to treatments described in Table 1.

Coating treatments were carried out by immersing the walnuts in each coating solution (Table 1) for 5 min. After that, the walnut kernels were drained and let to stand at room temperature (20 °C) until completely dry (almost 5 h). Then, an equal amount of treated (Coating and Coating-GGE) and untreated (Control) walnut kernels were placed

Table 1
Treatments of walnuts under study.

	Walnut treatments
Control	Uncoated
Coating	Treated with coating containing 1.5 g of chitosan +25 mL of pure glycerol and 75 mL of 1% glacial acetic acid solution
Coating-GGE	Treated with coating containing 1.5 g of Chitosan +25 mL of GGE and 75 mL of 1% glacial acetic acid solution

in a plastic container, incubated at 45 °C and evaluated after 2, 7 and 10 d for the parameters reported below.

2.4.1. Lipid oxidation and peroxide values

In order to verify if the described treatments (Table 1) can prevent the oxidation of the stored nuts, walnuts oil was extracted by cold extraction. In brief, for each treatment, 30 g of walnuts were grinded and the obtained flour was transferred to a 250 mL round neck flask; therefore, 50 mL of diethyl ether were added to the flask and stirring was applied using a magnetic stirrer for 30 min with subsequent 10 min of resting. After resting, the top layer was carefully removed and filtered into 250 mL flask. The extraction process was repeated two times, using 50 mL of petroleum ether and diethyl ether solution (1:1). Solvent was removed through rotary evaporation using rotary evaporator.

The walnut lipid oxidation was evaluated using the method reported by Gong et al. (2022). The assay, called also TBARS test because it involves the use of thiobarbituric acid reactive substances, was carried out mixing 0.30 g of extracted walnut oil with 6 mL distilled water, 9 mL of phosphoric acid (H_3PO_4 at pH 2.0) and 9 mL 0.8% (w/w) of thiobarbituric acid (TBA) in 1.1% (w/w) sodium dodecyl sulfate (SDS). The mixture was then vortexed and heated at 100 °C for 60 min in a water bath. After cooling, butan-1-ol (30 mL) was added and the mixed solution centrifuged at 10.000 g for 10 min in a centrifuge ALC 4239R (ALC, Winchester, VA, USA). The absorbance of the upper layer was measured at 532 nm using PerkinElmer lambda 25 UV-VIS spectrometer. A standard curve was created using the 1,1,3,3-TetraEthoxyPropane (TEP) and results expressed as mg TEP/kg sample.

Peroxide value of extracted walnut oil was evaluated using the method reported by Lin et al. (2022). Walnut oil (about 4 g for each sample under study) was placed into a 250 mL Erlenmeyer flask and opportunely mixed with 30 mL chloroform/glacial acetic acid solution (2:3 v/v) and 1 mL saturated potassium iodide (KI). The resultant solution was let to stand in the dark for 5 min and after that time 75 mL distilled water and 1 mL 1% soluble starch solution was added. Titration of the prepared solution was carried out using 0.002 mol/L sodium thiosulfate until the solution turns from blue to colorless.

Peroxide value (PV) was calculated following the formula:

$$PV = \frac{1000 * (\text{mL sodium thiosulfate used in blank} - \text{mL sodium thiosulfate solution used in sample})}{\text{g of walnut oil}}$$

PV was expressed as milliequivalents (meq) of free iodine per kilogram of oil (meq/Kg). For each treatment (Table 1) and at each time point (2, 7 and 10 d of storage) all the analysis described above were carried out on the 3 independent replicates.

2.4.2. Color parameters

Color of samples (Table 1) during storage (2, 7 and 10 d) was measured using a portable colorimeter Konica Minolta CM-2500d (Bremen, Germany), with an illuminant D65. Each sample was reduced to a homogeneous flour and evaluated through the accessory CR-A50.

CIE L*a*b* parameters lightness (L*), redness (a*) and yellowness (b*) were recorded and also used to calculate the ΔE, that is a parameter commonly investigated to highlight the "distinct" differences between sample. As reported by Anger (1977) the differences between samples can be considered "very distinct" if $\Delta E > 3$, "distinct" if $1.5 < \Delta E < 3$ and "without perceptible differences" if $\Delta E < 1.5$.

The ΔE parameter was calculated using the following Equation:

$$\Delta E = \sqrt{(L_x - L_0)^2 + (a_x - a_0)^2 + (b_x - b_0)^2}$$

where subscript "x" indicates the color of the sample coated with or without GGE and the subscript "0" indicates the color of the control sample.

Moreover, the parameter chroma (C*), representing the color intensity (McGuire, 2019) was evaluated. Each walnut sample of the 3 independent replicates was subjected to 2 readings, for a total of 6 readings for each treatment.

2.4.3. Antifungal activity

The antifungal potential of the coating-GGE, in preventing the growth of *A. flauvs* growth on walnuts, was evaluated using the method recently reported by Parafati et al. (2022), with minor modifications. Walnut kernels were sanitized by dipping the fruits into ethanol:water (70:30 v/v) solution for 5 min. After that, walnuts were air dried and then subjected to the treatments reported in Table 1.

Aspergillus flavus var. *flavus* strain CBS 573.65, was obtained from the Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre (Utrecht, The Netherlands). The mold stock culture was maintained on Potato Dextrose Agar (PDA; CM0139, Oxoid, Basingstoke, UK) at 4 °C and refreshed on PDA for 10 d at 26 °C previous its use in the experiments.

In brief, ten walnuts, for each treatment group, were wounded (one hole for each seed) with a sterile needle and then inoculated with 10 μL of *A. flavus* spore suspension (10^5 spores/mL). After air-drying, the fruits were placed in a sterile Petri plate, and stored at 25 °C and 85% relative humidity. Uncoated walnuts inoculated with *A. flavus* spore suspension served as control.

The *A. flavus* disease incidence (DI) was calculated as percentage of infected walnuts with visible presence of mycelium on total walnuts and recorded after 7 and 10 d of storage at 25 °C. Moreover, sporulation capacity of the fungus was calculated at the same time points and expressed as spore number produced in the infected walnuts, by transferring walnuts of each treatment group in a suitable quantity of sterile distilled water (SDW) with 0.2% Tween 80 (Merck) and shaking at 180 rpm for 4 h. Spores contained in water suspensions were then counted using Bürker chamber under optical microscope. The concentration of conidia was expressed as number of spores/g of walnut and quantified considering the initial weight of the walnuts and the amount of SDW

Table 2
Functional characterization of glycerol ginger extract (GGE).

Bioactive compounds	
Total polyphenols (mg/100 mL)	114.33 ± 17.68
Total flavonoids (mg/100 mL)	13.47 ± 2.06
Radical scavenging activity (%)	83.67 ± 3.1

used. Results were calculated using 3 independent replicates for each treatment.

2.5. Statistical analysis

Data obtained from the 3 independent replicates were analyzed independently and subjected to One-Way analysis of variance (ANOVA) in order to assess any significant difference among samples ($p < 0.05$). Fisher's least significant difference test was used to compare the significance of differences and performed used the statistical package software Minitab™ version 20.0.

3. Results and discussion

3.1. Bioactive compounds of ginger extract

Table 2 summarizes the content of bioactive compounds of GGE prepared as reported above. Results show that the GGE has a good content in total polyphenols, equal to 114.33 ± 17.68 mg GAE/100 mL, of which more than 10% is represented by flavonoids (13.47 ± 2.06 mg QE/100 mL). Although the total polyphenol content is slightly lower than that reported by other authors (Makanjuola & Enujiugha, 2017; Tanweer et al., 2020), the GGE presents a greater content in flavonoids in comparison to other research studies (Mushtaq et al., 2019). The RSA obtained was equal to 83.67 ± 3.1%, a value similar to those reported by Sharif and Bennett (2016) of 87.1% and 82.2%, but using acetone and methanol as solvent, respectively.

The RSA% could be directly correlated to the presence of high amount of flavonoids as affirmed by Agati et al. (2020) and Sungpud et al. (2020), who described the glycerol as second-best extraction solvent after propylene glycol in bio-based extraction solvents. Glycerol has also been quoted as a potential solvent for the extraction of bioactives from other vegetable matrix as reported by Kowalska et al. (2021), who highlighted the efficiency of this solvent in the extraction of such compounds from black chokeberry and elderberry fruits.

3.2. Evaluation of coated walnut kernels under storage

3.2.1. Lipid oxidation and peroxide value

The efficacy of coating carrying GGE, in preventing lipid oxidation and the increase of peroxide on walnut kernels stored at 45 °C, was evaluated up to 10 d of storage.

Immediately after shelling, walnut samples evidenced a value equal to 9.15 ± 0.10 mg TEP/Kg of oil. As displayed in Fig. 1, after 2 d of incubation, the oxidation of lipids increased in all samples under study (Table 1) and in particular in uncoated walnuts (Control) that registered the significantly ($p < 0.05$) highest value of 12.48 ± 0.40 mg TEP/Kg. If compared to the Control, both coating treatments (Coating and Coating-GGE) let down the lipid oxidation of walnuts; the significantly ($p < 0.05$) lowest lipid oxidation was recorded in Coating-GGE walnuts that showed the value of 10.14 ± 0.11 mg TEP/Kg.

Similar effects were observed during considered storage time, demonstrating the efficacy of the Coating-GGE treatment. Walnut thus treated, in fact, evidenced the significantly ($p < 0.05$) lowest lipid oxidation values during all the storage period (Fig. 1).

Concerning the peroxide value (PV), the initial number of peroxides determined in walnuts, immediately after shelling, was below the detection limits of the method, but it increased during storage at 45 °C. Fig. 2 displays the change in PV of walnuts after 2, 7 and 10 d of storage.

After 2 d of storage, all samples evidenced an initial oxidative rancidification, which resulted in an increase of PV to the values of 0.99 ± 0.06, 1.26 ± 0.07 and 0.59 ± 0.13 ($meqO_2$/Kg of oil), respectively for Control, Coating and Coating-GGE samples. This latest evidenced the significantly ($p < 0.05$) lowest PV after 2 d and even after 7 and 10 d of storage. In fact, during storage, the PV increased drastically in the Control sample, but very moderately in the sample treated with the Coating-GGE that, at the end of the storage period (10 d), recorded halved PV compared to the Control (Fig. 2).

As already reported from different authors, the use of chitosan coating is effective in preventing oxidation of walnuts, probably acting as oxygen barrier (Sabaghi et al., 2015; Siripatrawan & Harte, 2010). The treatment of walnuts with Coating-GGE allowed to obtain a more noticeable reduction, even compared to chitosan Coating alone, in the oxidation of the product and therefore in the development of its rancidity, thanks to the great antioxidant activity of the GGE. Similar results in inhibiting walnuts oxidation were reported by Sabaghi et al. (2015), who investigated the effect of green tea extract incorporated into chitosan-based coating.

3.2.2. Color evaluation

Table 3 evidences the color parameters of walnuts differently treated (Table 1) and stored at 45 °C for 10 d. Before storage, walnuts samples, prepared as described in material and methods section, evidenced the following color values: 48.9 ± 1.7, 1.7 ± 0.4 and 13.9 ± 0.9 recorded respectively for L*, a* and b*.

The chroma value (C) of the color came up with a peculiar trend showing slight and inconsistent changes during the first 7 d of storage. After 10 d of storage, both Coating and Coating-GGE samples evidenced

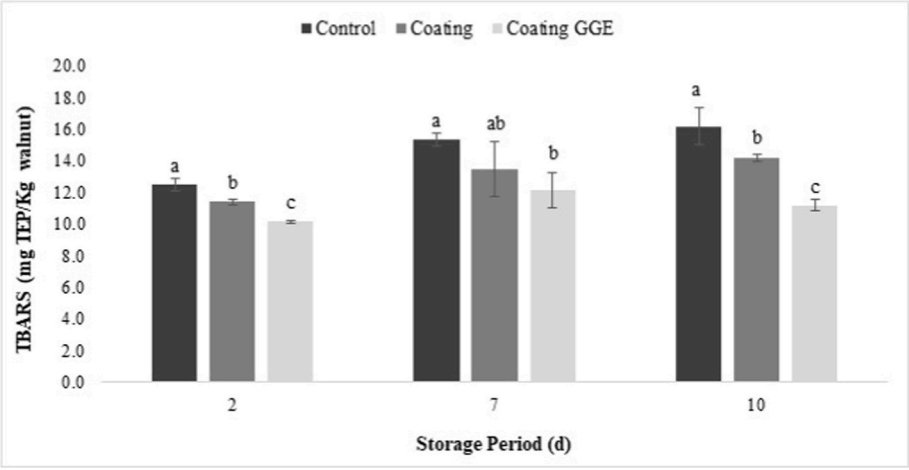

Fig. 1. Lipid oxidation of treated samples under study (Table 1) evaluated after 2, 7 and 10 d of storage at 45 ± 1 °C. Data are represented as means of three replicates ± standard deviation. Within each time point (2, 7 or 10 d), values with different letters are significantly different ($p < 0.05$), according to Fisher's least significant difference test.

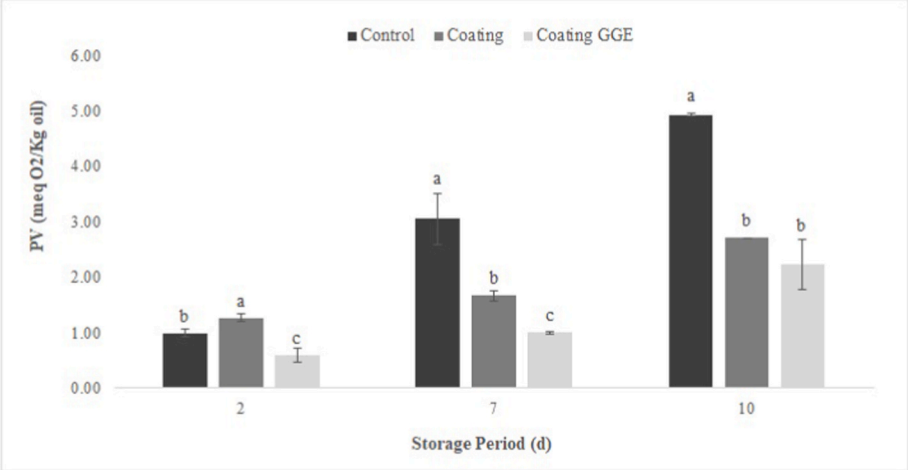

Fig. 2. Peroxidation value (PV) of treated samples under study (Table 1) evaluated after 2, 7 and 10 d of storage at 45 ± 1 °C. Data are represented as means of three replicates ± standard deviation. Within each time point (2, 7 or 10 d), values with different letters are significantly different ($p < 0.05$), according to Fisher's least significant difference test.

Table 3
Color parameters of treated walnuts during storage.

Storage time (d)		Color parameters				
	Treatments	L*	a*	b*	C	ΔE
2	Control	41.46 ± 1.4a	1.38 ± 0.2b	9.46 ± 1.0a	9.60 ± 1.0a	
	Coating	39.44 ± 1.2b	1.78 ± 0.2a	8.79 ± 0.5a	9.00 ± 0.5a	2.26[a]
	Coating-GGE	43.05 ± 1.1a	1.47 ± 0.2b	8.78 ± 0.7a	8.90 ± 0.7a	1.64[b]
7	Control	42.39 ± 1.1a	1.61 ± 0.2a	8.14 ± 0.4a	8.30 ± 0.4a	
	Coating	40.00 ± 1.2b	1.58 ± 0.1a	8.23 ± 0.6a	8.40 ± 0.6a	2.39[a]
	Coating-GGE	40.56 ± 1.3b	1.59 ± 0.2a	8.40 ± 1.0a	8.60 ± 1.0a	1.85[b]
10	Control	40.69 ± 2.3a	1.60 ± 0.1c	8.29 ± 0.8b	8.44 ± 0.8b	
	Coating	37.56 ± 0.6b	2.88 ± 0.2a	9.27 ± 0.0a	9.71 ± 0.1a	3.52[a]
	Coating-GGE	40.26 ± 0.6a	1.93 ± 0.1b	9.29 ± 0.2a	9.50 ± 0.2a	1.13[b]

Data presented as mean ± standard deviation of the mean. In each column, within the same storage time (2, 7 and 10 d), values followed by different letter are significantly different according to Fisher's least significant difference test ($p < 0.05$). Within each storage time (2, 7 or 10 d).
[a] Indicate data regarding differences (ΔE) between control sample and sample treated with Coating.
[b] Indicate data regarding differences (ΔE) between Control sample and sample treated with Coating-GGE.

a significant ($p < 0.05$) increase in chroma, a* and b* values in comparison to the Control (Table 3).

Considering the ΔE, when each treated sample, Coating or Coating-GGE, was compared with the uncoated walnuts (Control) the differences were "distinct" after 2 and 7 d of storage, assuming in fact, values corresponding to $1.5 < \Delta E < 3$. After 10 d of storage sample treated with Coating evidenced "very distinct" differences in comparison to the Control ($\Delta E > 3$), while "no perceptible differences" were detected between the Control and the sample treated with Coating-GGE ($\Delta E < 1.5$).

3.2.3. Antifungal activity of Coating-GGE

Table 4 reports the disease incidence (DI) of *A. flavus* on walnuts differently treated as reported in Table 1.

After 7 d of incubation at 25 °C, the Control sample evidenced the highest DI% of 50.7 ± 16.7, followed by sample treated with Coating that registered the value of DI% equal to 16.7 ± 0.0. In comparison to the Control, the use of the Coating treatment was effective in reducing the DI%, despite the best result was obtained in sample treated with Coating-GGE where no fungal growth was observed (Table 4).

After 10 d of storage, Control walnuts evidenced 100% of DI and even the highest number of spores (6.36 ± 0.16 Log/g of sample), whereas, the sample Coating-GGE registered the significantly ($p < 0.05$) lowest value of DI% (17.0 ± 0.0) and number of spores (1.36 ± 0.07 Log/g of sample).

The obtained results were certainly due to the combined antifungal effect of chitosan coating (Bhaskara Reddy et al., 2000; Campaniello et al., 2008; Maghsoudlou et al., 2012; Romanazzi et al., 2006) and to the bioactive compounds of the GGE. Due to its chemical characteristics, the employment of glycerol probably allows to extract a good quantity of ginger oil that, as reported by Kalhoro et al. (2022), is rich in citral, a chemical constituent responsible for the antifungal activity of ginger. Moreover, phenolic compounds (eugenol, shogaols, zingerone, gingerdiols, gingerols, etc.) and their synergistic relationship with other compounds such as β-sesquiphellandrene, *cis*-caryophillene,

Table 4
Inhibition of *A. flavus* growth on treated walnuts.

Treatments	*A. flavus* inhibition			
	Storage time 7 d		Storage time 10 d	
	Disease Incidence (%)	Number of spores (Log/g of sample)	Disease Incidence (%)	Number of spores (Log/g of sample)
Control	50.7 ± 16.7a	6.04 ± 0.03a	100 ± 0.0a	6.36 ± 0.16a
Coating	16.7 ± 0.0b	4.67 ± 0.00b	72.2 ± 9.6b	5.67 ± 0.12a
Coating-GGE	0.0 ± 0.0c	0.00 ± 0.00c	17.0 ± 0.0c	1.36 ± 0.07b

Data presented as mean ± standard deviation of the mean. In each column, within the same parameter (Disease Incidence and Number of spores), values followed by different letter are significantly different according to Fisher's least significant difference test ($p < 0.05$).

zingiberene, α-farnesene, α- and β-bisabolene, have been recognized as mainly responsible for the antimicrobial activity found in ginger essential oil, extracts, and oleoresins (Del Carmen Beristain-Bauza et al., 2019 and references therein).

4. Conclusions

The use of Coating-GGE has allowed to obtain a significant ($p < 0.05$) reduction of walnut oxidation. In particular, it has been noted that, in comparison to Control sample and sample treated only with chitosan Coating, the walnuts coated with Coating-GGE evidenced the lowest lipid oxidation and PV during all the storage period (10 d at 45 °C). Moreover, Coating-GGE has proven to be effective in reducing the growth of *A. flavus*, a really dangerous pathogen that causes huge amount of post-harvest losses and can potentially produce carcinogenic mycotoxin, the aflatoxin B_1. The results obtained in this study show interesting perspectives for improving the nutritional and microbiological quality of nuts, as well as their safety and shelf life, although the advantages of applying Coating-GGE could also be exploited in other food matrices subject to fat rancidity. Further studies will be carried out, even using different ginger concentrations, in order to assess the efficacy of Coating-GGE against *A. flavus* aflatoxin production and to verify the final acceptability of the treated walnuts through sensory tests.

Author Contributions

Conceptualization, C.R., R.P. and B.F.; methodology, R.P., L.P. and C. R.; formal analysis, M.N.S. and L.P.; writing–original draft preparation, M.N.S., L.P. and C.R.; writing–review and editing, L.P. and C.R.; supervision, B.F. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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