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Potential of nanochitosan coating combined with walnut green husk to improve the preservation of rainbow trout (*Oncorhynchus mykiss*) during refrigerated storage

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ABSTRACT

The present study evaluated the potential of nanochitosan coating enriched with ethanol-water extract of the walnut green husk (WGHE) on spoilage and rancidity of rainbow trout (*Oncorhynchus mykiss*) during six-day refrigerated storage. Hence, we have considered fresh trout fillets without any treatment as control (C), immersed in 2% solution of chitosan nanoparticles (CN), combination with 1.5% and 3% WGHE with nanochitosan coating (CN + WGHE 1.5 and CN + WGHE3), for physicochemical, microbial and sensorial assays. The highest levels and total volatile nitrogen were observed after day 6 in C, while the lowest was found in CN + WGHE3 groups. Thiobarbituric acid reactive substance (TBARS) and peroxide value of untreated fillets on day 6 of the study were significantly higher than NC + WGHE3 with 0.08 mg/g and 3.27 mEq/kg, respectively. The total microbial population was: C > CN > CN + WGHE 1.5 > CN + WGHE3, which expresses the effect of the extract on the total microbial population. Overall, the combination of WGHE with CN increased the extract's efficiency in reducing peroxide value, TBARS, and total volatile nitrogen and delayed the pH increase, improving the overall acceptability of rainbow trout fillets stored in refrigerated conditions.

1. Introduction

Rainbow trout (*Oncorhynchus mykiss*) is considered a great source of protein, minerals, vitamins, and long-chain polyunsaturated fatty acids (LC-PUFAs) (Abedi et al., 2016), hence its consumption is associated with a healthy and balanced diet. However, its commercial availability presents some weak points. Compared to other fresh products, fish is a highly perishable food. Due to large volumes of LC-PUFA omega-3 (ω3), they are very disposed to oxidative spoilage and rancidity (Abedi et al., 2016). Several factors, including the level of water activity, pH, free amino acids, unsaturated fatty acids, and the content of protein and enzymes, are involved in fish deterioration. In addition, various biological reactions such as oxidation of lipids, protein degradation, or decomposition mediated by endogenous or microbial enzymes cause their spoilage (Jeyasekaran et al., 2012). Which finally tend to shorten their shelf life and other seafood products (Hosseini et al., 2016). As a result, the alterations in organoleptic properties of fish mainly result from these oxidative reactions (Naseri and Rezaei, 2012; Tavakoli et al., 2018).

Using low temperature is among the most common methods to preserve fish, theoretically hindering undesirable changes; however, the reduction in quality may occur even under these conditions (Jeon et al., 2002). Hence, many researchers are recently focused on plant extracts and essential oils as alternative bio-preservatives in the food industries

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and processing (Abedi et al., 2016; de Carvalho and Conte-Junior., 2021). The inhibitory effects of plant extracts on the microorganisms are long known; for instance, grape seed and clove (Shi et al., 2014), marjoram essential oil (Hosseini et al., 2016; Mexis et al., 2009), cinnamon oil (Andevari and Rezaei, 2011; Zhang et al., 2017), salvia and clove essential oil (Çoban et al., 2014), extract of Trachelospermum and Persian shallot seeds (Raeisi et al., 2016), and extract of pomegranate husk; all have presented positive influence in preventing the undesirable reactions involved in fish spoilage (Berizi et al., 2018; Zarei et al., 2015). Applying chitosan solely or enriched with other active ingredients could enhance the storage time microbial and oxidative stability of fish (Oladzadabbasabadi et al., 2022; Homayonpour et al., 2021; Mehdizadeh et al., 2021) and lamb meat (Pabast et al., 2018). Also, many studies have introduced nanoparticles technology to affect microorganisms, which resulted in prolonging the shelf life of foods (Jafarzadeh et al., 2017, 2022; Zarei et al., 2015). Chitosan (Li et al., 2012; Berizi et al., 2018; Hassanzadeh et al., 2018; Yu et al., 2018) and nanochitosan coatings have also been used to extend the shelf life of fish under different storage conditions (Mehdizadeh et al., 2021; Homayonpour et al., 2021; Ramezani et al., 2015; Tapilatu et al., 2016; Zarei et al., 2015), which usually enhance the antimicrobial and antioxidant activities of the extracts, when used simultaneously (Ojagh et al., 2010; Zarei et al., 2015; Yuan et al., 2016; Berizi et al., 2018; Yu et al., 2018).

Walnut (Juglansregia L.) is a valuable crop and widely grown worldwide. The major producers of walnut, as very popular and widely consumed nut, are China, the United States of America, and Iran, attributed with 25%, 20%, and 11% of total global production, respectively (Shah et al., 2021). The industrial applications of different parts of green walnut, including shells, kernels, barks, green walnut husks (epicarp), and leaves, have been previously reported (Pereira et al., 2007; Gharibzahedi et al., 2014) In addition, parts of the walnut are considered valuable as a wide range of healthcare compounds, and have been intensively used due to their antimicrobial properties (Akbari et al., 2012; Stampar et al., 2006), anti-inflammatory effects (Jiménez-Gómez et al., 2009), antifungal (Pereira et al., 2007) and antioxidant activity, as well as their high amounts of total phenolic compounds (Jakopic et al., 2007; Jahanban-Esfahlan et al., 2019).

The walnut's green husk extract (WGHE) has shown significant antimicrobial properties, especially against gram-positive bacteria (Oliveira et al., 2008). Furthermore, our studies have emphasized the richness of WGH in terms of bioactive compounds, which enables this byproduct to be used as a preservative in meat products (Cosmulescu et al., 2010; Akbari et al., 2012; Fernández-Agulló et al., 2013; Salejda et al., 2016). Considering these properties, WGH has the capabilities to be potentially used as a natural preservative for vulnerable food such as meat and fish. Furthermore, the total phenolic content (TPC) of WGHE in different walnut species has been investigated by aqueous extraction, and 13 phenolic compounds have been identified (Stampar et al., 2006). The scavenging activity of DPPH radicals (2,2-diphenyl-1-picrylhydrazyl) at the concentration of 1 mg/mL of green husk extract was 83.16%–93.92%. Hence, the utilization of the walnut's green husk byproduct (WGH), i.e., the "non-edible" part, is considered as a potential source of natural bioactive compositions with potent antioxidant properties, which can exert inhibitory effects on the growth of various pathogenic bacteria, which will additionally present an opportunity for the valorization of this byproduct (Carvalho et al., 2010; Oliveira et al., 2008).

This investigation studied the increased refrigerated storage of rainbow trout by applying chitosan loaded with walnut husk extract. To the best of the authors' knowledge, this is the first study to synthesize chitosan nanoparticles with walnut green husk extracts as a combined method for fish preservation. The physicochemical properties (phenolic content, flavonoids, peroxide, pH, malondialdehyde, volatile base-nitrogen), microbial load (total and psychrophiles), and sensorial properties (panel evaluation of color, odor, appearance, and acceptance) of stored rainbow trout during 6-day refrigerated storage were assessed. With these three levels, the suitability of the combined intervention was scrutinized, aiming to provide a new method for fresh food preservation during cold storage.

2. Materials and methods

2.1. Materials

Iranian walnut green husk was prepared in the chemical laboratory of Yasuj University of Medical Sciences. Chitosan (450 kDa; 75–85% deacetylation), sodium tripolyphosphate, Folin-Ciocalteu reagent, sodium carbonate, 2,2,1-diphenyl-1-picrylhydrazyl (DPPH), acetic acid, magnesium oxide, sulfuric acid, boric acid, thiobarbituric acid (TBA), malondialdehyde dichloromethane, and ethanol were provided by Sigma-Aldrich. Plate count agar (PCA) was obtained from Merck..

2.2. Sample preparation

Iranian walnuts (from Southwestern Iran) were harvested directly from trees with the same genotype and aging. Green walnuts were picked manually, randomly, and with no damage to the green husk. The green husk was separated from collected fruits manually on the same day of harvesting and was dried at room temperature, milled, then sieved on Mesh-20 to a fine powder. This powder is stored in brown glass containers kept at −18 °C in order to protect its antioxidant and antimicrobial properties.

2.3. Preparation of the walnut green husk extract (WGHE)

To prepare the 50% v/v of the ethanol-water extract of WGH, 1.5 g of WGH powder was dissolved in 25 mL of solvent for 45 min at room temperature and was then filtered through Whatman No.4 filter paper. The solvent was evaporated under a vacuum, and the extract was redissolved in water to a final concentration of 50 mg/mL. The extract (WGHE) was kept at 4 °C in dark bottles (Fernández-Agulló et al., 2013). The optimum concentrations of WGHE in a 2% chitosan nanoparticle solution was initially prepared.

2.4. Preparation of chitosan nanoparticles

According to the method developed by Pabast et al. (2018), chitosan solution was prepared with some modifications. Briefly, 2% coating solution (w/v) was obtained by dissolving chitosan powder (with a molecular weight of 450 kDa; 75–85% deacetylation degree, Sigma-Aldrich Chemical Company, USA) in 1% (v/v) acetic acid. The solution was blended (350 rpm) for 1 h at 90 °C to gain complete dispersion of chitosan. To eliminate the insoluble parts, the solution was then filtered through a Whatman No. 3 filter paper. In addition, 2% (w/v) sorbitol was added as a plasticizer.

In order to prepare the chitosan nanoparticles, the ionotropic gelation between the chitosan and sodium tripolyphosphate method was applied. A 2% sodium tripolyphosphate solution in water was prepared and mixed using magnetic stirring at room temperature. 4 mL of 2% tripolyphosphate solution was added to 100 mL of 2% chitosan solution. The mixture was shaken for 60 min and then subjected to 1.5 kW ultrasonic homogenization process for 10 min (Sonoplus, Bandelin electeronic, Germany). Zetasizer Nano-Zs-90 software was employed to analyze the particle size and Zeta Potential (Malvern Instruments, Malvern, UK) at a 90° scattering angle at 25 °C. The samples were dispersed in 0.1 mM KCl and measured automatically for the Zeta Potential measurements (Zarei et al., 2015).

2.5. Raw fish, preparation, and processing

A total of 60 freshly rainbow trout (*Oncorhynchus mykiss*), with an average weight of 550–650 g, were provided by a fish farm in Shiraz,

which were transferred immediately to the laboratory at the Research Center for Health Sciences, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran. The fish were then washed, and viscera, head, and bones were removed manually; two fillets of each fish were prepared (varying from 35 to 55 g in weight and 0.7–1 cm in thickness). Fillet samples were randomly divided into four treatment groups consisting of: one control (C); immersed in distilled water, two CN; coated in chitosan nanoparticles solution of 2%, three CN + WGHE1.5; treated with 2% chitosan nanoparticles solution with 1.5% ethanol-water extract of the walnut green husk and four CN + WGHE3; coated in chitosan nanoparticles solution of 2% with 3% WGHE. Each treatment performed in triplicate and the mean values were used to analysis. For coating process, each fillet sample was immersed for 2 min in 400 mL of the chitosan nanoparticles and WGHE solution and then rinsed well on a pre-sterilized metal net. Cellophane was used to pack the samples before being stored in the refrigerator at 4–7 °C for evaluation during a maximum of 6 days of storage with 3-day intervals.

2.6. Total phenolic content of WGHE

The Folin-Ciocalteu reagent (FCR) was used to assess the total phenolic content (TPC) of the ethanol-water WGHE based on the method of Singleton and Rossi (Singleton and Rossi, 1965). Briefly, 6 mL of distilled water and 500 μL of Foli-Ciocilteu reagent (Sigma–Aldrich, USA) were added to 100 mL of WGHE, mixed thoroughly, and kept at room temperature for up to 8 min 1.5 mL of sodium carbonate (20% w/v) and 1.9 mL of distilled water were then added to neutralize the reaction mixture. The extract was shaken in a warm water bath at 40 °C for 30 min. The absorbance of the solution was recorded at the wavelength of 765 nm using a UV–visible spectrophotometer (Model: UV–VIS 1700 Metrolab, Argentina). A mixture of water and Foli-Ciocilteu reagent was used as the negative control. The gallic acid standard curve concentrations of phenolic compounds were obtained and reported as the mean [mg of gallic acid equivalents (GAEs) per 100 g of sample] ± Standard Deviation, using triplicate extracts.

2.7. Total flavonoid content assay

To measure the total flavonoid content of WGHE, a modified colorimetric method was used (Yang et al., 2009). Briefly, 1.25 mL of distilled water and 0.07 mL of sodium nitrite solution (5%, w/v) were mixed to prepare with 1:10 diluted extracts (0.25 mL) and allowed to react for 5 min. Then, 0.15 mL of aluminum chloride solution (10%, w/v) was added to the mixture and allowed to react for further 6 min before the addition of 0.5 mL of NaOH (1 M). 1 mL milli-Q (ultrapure) water was then added to the samples. The absorbance of the mixture was read at the wavelength of 510 nm (Model: UV–VIS 1700 Metrolab, Argentina). The catechin standard curve was used to determine the triplicate extracts flavonoid content which was then stated as the mean [mg catechin equivalents (CEs) per 100 g of sample] ± standard deviation for the triplicate extracts.

2.8. Antioxidant activity

2,2,1-diphenyl-1-picrylhydrazyl (DPPH) was used to calculate the antioxidant activity by a radical scavenging method, as described by Berizi et al. (2018). The WGHE (50 mL) was added to 200 μL of DPPH ethanol solution at a concentration of 0.1 mmol/L. The solution was gently mixed and left at room temperature in the dark for 30 min. The absorbance of the obtained solution was measured at 515 nm with 5 min time intervals using a UV–visible spectrophotometer (Model: UV–VIS 1700 Metrolab, Argentina) until its stabilization. The activity of DPPH radical scavenging was calculated as DPPH discoloration, while DPPH without any sample was used as a control.

2.9. Measurement of peroxide value (PV)

The method of Woyewoda et al. (1986) was used to record the peroxide content of samples. The results were presented as mill equivalent of peroxide per 100 g of fat.

2.10. Thiobarbituric acid (TBA) measurement

Method of Mielnik et al. (2006), with some modifications, was used to measure TBA levels. The TBA content of samples was expressed as mg MDA (kg fish^{-1}) regarding 1, 1, 3, 3-teraethyoxy propane standard curve.

2.11. pH measurement

The pH value of fish samples was directly calculated using a pH meter electrode (CG824, Germany) at room temperature.

2.12. Total volatile base-nitrogen (TVB-N)

The micro-diffusion technique was used to determine the value of TVB-N (Goulas and Kontominas, 2005). Two gram of magnesium oxide (MgO) was mixed in 10 g homogenized fish samples, which were finally adjusted into 400 mL distilled water. A 2% aqueous boric acid solution was used to collect the distillate, which was titrated using 0.05 mol/L sulfuric acid solution in the presence of methyl red–methylene blue indicator as to the endpoint detection sign. TVB-N content of each sample was then reported as mg N/100 g of fish.

2.13. Microbial analysis

90 mL of 0.1% peptone salt solution (NaCl, 0.85%, w/v) was used to homogenize (Heidolph, Germany) the samples (10 g of each) for 1 min with a stomacher. The samples were subsequently serially diluted tenfold using 0.1% peptone water, then sub-cultured onto Plate Count Agar (PCA, Merck, Germany) to enumerate total aerobic bacteria. The enumeration of total viable count (TVC) was completed following incubation at 37 °C for two days, and viable psychrophilic count (PVC) was performed following incubation (Memmert, Germany) at 15 °C for seven days. Data were expressed as log CFU/g (Sallam, 2007).

2.14. Sensory evaluation

Ten trained panelists assessed the fish samples based on the Berizi et al. (2018) method. A 5-score scale unit (1 very poor to 5 excellent) evaluated color, odor, appearance, and overall acceptance of rainbow trout fillets was employed. The collected results from the questionnaire were statistically evaluated in particular tables. Finally, the mean score given by individuals in each iteration was calculated and reported for the product. The study protocol received ethical approval from the Research Ethics Committee of Yasuj University of Medical Sciences.

2.15. Statistical analysis

The SPSS software version 16 was used to analyze the data. Details of data analysis in triplicate are given (as mean ± standard errors). The results are expressed as Duncan's multiple range test (DMRT) at $p < 0.05$ following analysis of variance (ANOVA). Different letters in figures and tables represent significant differences between groups ($P < 0.05$).

3. Results

3.1. Particle size and zeta potential of chitosan nanoparticles

The diameter of chitosan nanoparticles was 163 ± 6.4 nm, and the mean zeta potential was 33.63 mV. Due to the minimum requirement of

30 ± mV zeta potential for the physical stability of the nanosuspension, we confirm that the chitosan nanoparticles were adequately prepared.

3.2. Total phenolic compounds and flavonoids in WGHE

Total phenolic compounds were equivalent to 48.3 ± 3.08 mg/mL of gallic acid (GAE) in extract and per gram of dry matter of WGHE. The concentration of flavonoids was 3.82 ± 0.7 mg/mL of catechin.

3.3. Antioxidant activity of WGHE

The ability to scavenge 2-diphenyl-1-picryl hydroxyl radicals (DPPH) is presented in Fig. 1. A simultaneous increase in the antioxidant activity with WGHE increase was observed ($P < 0.05$). The concentrations of 0.015, 0.031, 0.062, 0.012 mg/mL of WGHE with DPPH radical scavenging activity percent were 20.66, 54.93, 63.45 and 80.06% respectively.

3.4. The effect of WGHE and CN on peroxide value changes

The changes in peroxide value (PV) in fish fillet samples treated with two different concentrations of WGHE and nanochitosan are shown in Fig. 2A. This index increased significantly with rainbow trout storage time in all studied groups ($P < 0.05$). A significant difference was found between the four experimental groups and two evaluated fish storage times ($P < 0.05$). The highest and lowest peroxide value on day 3 of the study was related to C and CN + WGHE3 (6.33 ± 0.155 and 4.515 ± 0.091) groups as well as on day 6; the highest and lowest PV was related to C and CN + WGHE3, respectively (9.83 ± 0.042 and 6.56 ± 0.084 mEq/kg).

3.5. The effect of WGHE and CN on TBA content

The effect of WGHE at two different concentrations and nanochitosan coating on TBARS content of rainbow trout during refrigerated storage is presented in Fig. 2B. The TBARS content of fish samples on day 0 was 0.4807 ± 0.03125 mg malondialdehyde per gram of sample. With increasing storage time in the refrigerator, this index increased in all samples: On day 3 of rainbow trout storage in the refrigerator, the values of TBARS were not significantly different in the experimental groups ($P < 0.05$), however, on day 6 of storage significant differences were observed in CN + WGHE3, C and CN groups as well as CN + WGHE1.5 and C groups. The lowest and highest levels of TBARS on day 6 were for CN + WGHE3 and control groups, respectively (0.5596 ± 0.51138 and 0.6479 00 0.00955 mg/g, respectively) ($P < 0.05$).

3.6. The effect of WGHE and CN on pH changes

The changes in pH for the stored fish fillets are shown in Fig. 3. The pH of the samples increased with prolonging storage in the refrigerator. The pH in the control group increased significantly from 6.94 on day 0 to the highest amount, 7.47, on day 6. ($P < 0.05$). The lowest pH on day six was found in the CN + WGHE3 group, followed by CN + WGHE1.5, CN, and C groups, respectively. The changes in pH in control and nanochitosan groups were significant ($P < 0.05$) during fish storage in the refrigerator, whereas these changes were insignificant in two groups of CN + WGHE1.5 and CN + WGHE3 with chitosan, respectively ($P>0.05$).

3.7. The effect WGHE and chitosan nanoparticles on TVB-N content changes

The recorded index of TVB-N of different experimental groups throughout the storage is given in Fig. 4. A significant increase in TVB-N contents was noted among all the groups during storage (Fig. 4, $P < 0.05$). A TVB-N value was recorded as 35.45 ± 1.08 in the control group, while those of CN, CN + WGHE1.5, and CN + WGHE3 were 30.43 ± 0.25, 19.345 ± 1.42 and 18.28 ± 1.08 respectively on day 6. TVB-N values showed significant differences ($P < 0.5$) depending on the nature of treatments and storage times. A significant increase was found in TVB-N contents of C and CN samples over the storage time ($P < 0.05$), whereas this increase was insignificant in CN + WGHE groups in storage time between days 3 and 6 ($P < 0.05$).

3.8. The effect of WGHE on microbiological changes of stored rainbow trout in refrigerator

3.8.1. The effect of WGHE and chitosan nanoparticles on the population of psychrophilic bacteria

The effect of using WGHE and nanochitosan on the population of psychrophilic bacteria during refrigerator storing is shown in Fig. 5A. The results showed that the population of psychrophilic bacteria increased significantly on day 3 in all groups ($P < 0.05$), with the highest change in the control group. The lowest value was observed in the CN + WGHE3 group (3.33 log CFU g^{-1}). The results indicated that the psychrophilic bacteria population was significantly higher in the control group and significantly lower in the CN + WGHE3 group. This trend in the variation of PVC was also observed between days 3 and 6, and the number of psychrophilic bacteria increased significantly in all groups ($P < 0.05$), but the CN + WGHE treated samples presented lower concentrations of bacteria.

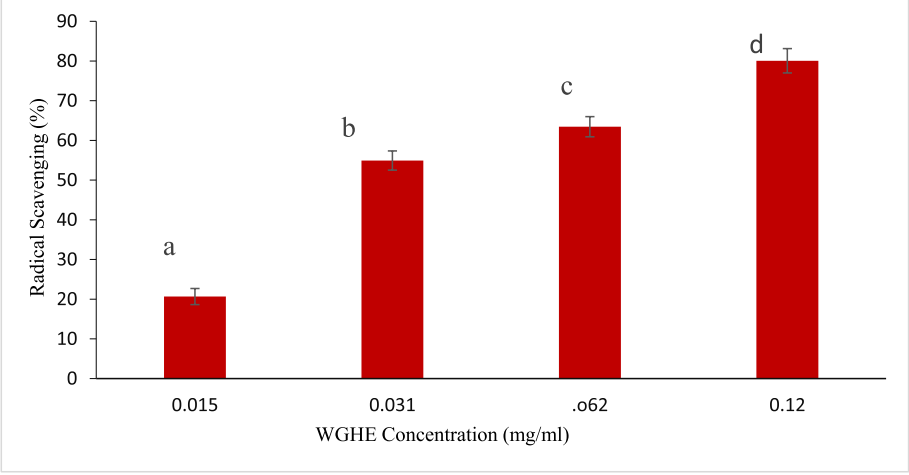
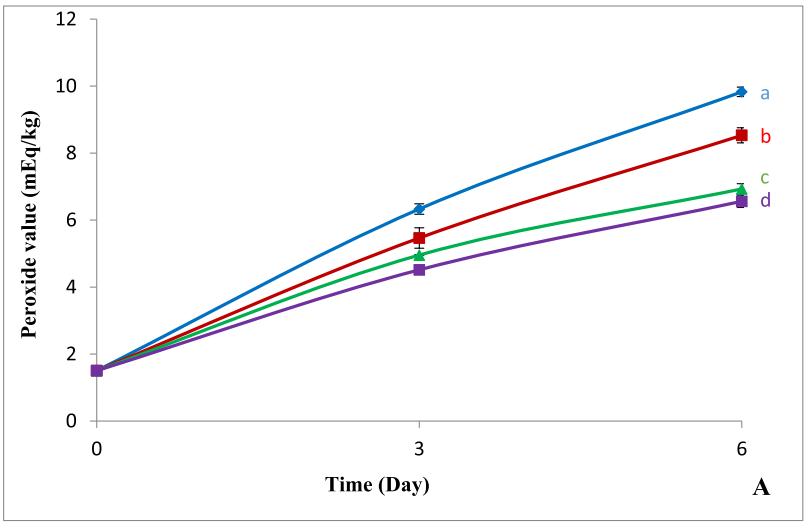


Fig. 1. Effect of different walnut green husk extract (WGHE) on DPPH radical scavenging. Different letters represent the significant difference ($P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



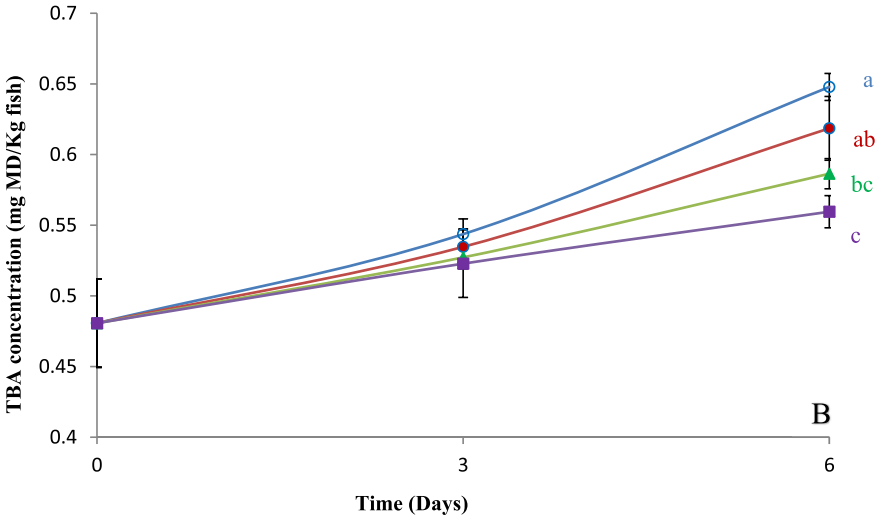


Fig. 2. Peroxide value (A) and TBARS (B) of rainbow trout fillet treated with chitosan nanoparticles containing walnut green husk extract (WGHE) over six days of storage in the refrigerator (means ± standard errors). ○: Control, ●: chitosan nanoparticles, ▲: chitosan nanoparticles +1.5% WGHE, ■: chitosan nanoparticles +3% WGHE. Different letters represent significant differences between groups on day 6 ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

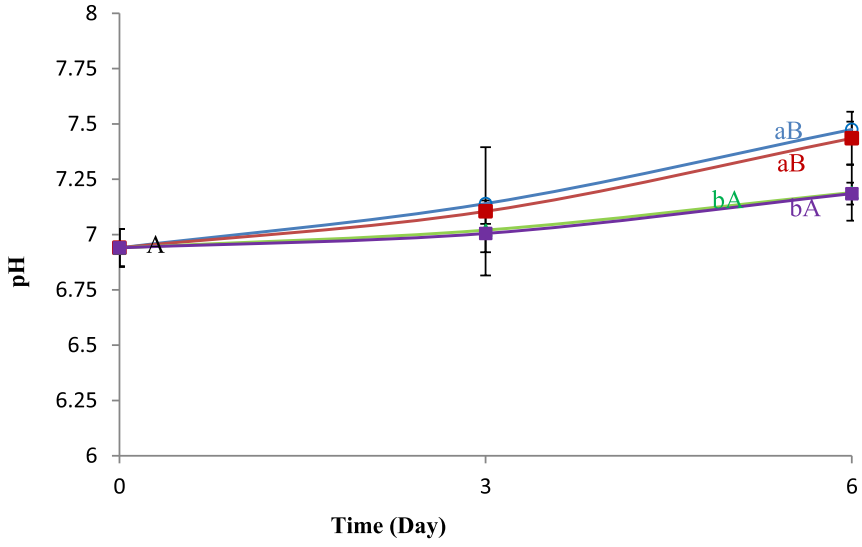


Fig. 3. pH of rainbow trout fillet treated with chitosan nanoparticles containing walnut green husk extract (WGHE) over six days of storage in the refrigerator (means ± standard errors). ○: Control, ●: chitosan nanoparticles, ▲: chitosan nanoparticles +1.5% WGHE, ■: chitosan nanoparticles +3% WGHE. Different uppercase and lowercase letters represent significant differences between days 0 and 6 and between groups on day 6, respectively ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

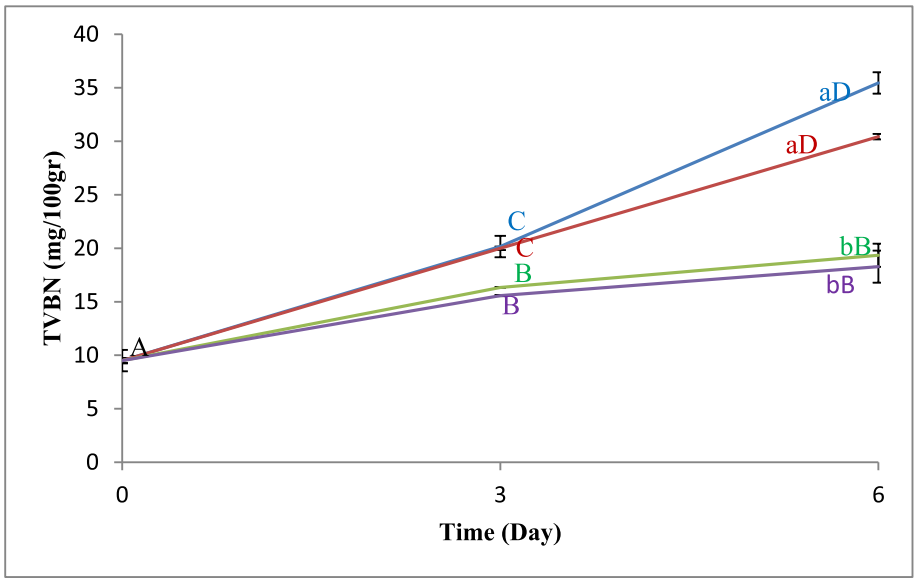


Fig. 4. TVB-N of rainbow trout fillet treated with chitosan nanoparticles containing walnut green husk extract (WGHE) over six days of storage in the refrigerator (means ± standard errors). ○: Control, ●: chitosan nanoparticles, ▲: chitosan nanoparticles +1.5% WGHE, ■: chitosan nanoparticles +3% WGHE. Different letters represent significant differences between study days ($p < 0.05$). Different uppercase and lowercase letters represent significant differences between days of study and between groups on day six, respectively ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.8.2. The effect of WGHE and nanochitosan on the total count of bacteria

The total viable counts (TVCs) of stored rainbow trout in the refrigerator treated with WGHE are shown in Fig. 5B. Over time, the total microbial population in all groups was increased significantly ($P < 0.05$). On both 3 and 6 days of storage, the TVC showed the following pattern: C > CN > CN + WGHE1.5 > CN + WGHE3. TVC of rainbow trout in control and nanochitosan groups was significantly higher than the two groups treated with WGHE ($P < 0.05$).

3.9. The effect of WGHE and nanochitosan on fish tissue sensory properties

The effects of WGHE on the sensory properties of stored rainbow trout in the refrigerator are given in Table 1. The organoleptic changes, including color, odor, and overall acceptability on day 0, did not differ significantly between all experimental groups ($P < 0.05$). Based on the evaluation by the experiment panelists, the organoleptic properties such as color and odor of fish were not significantly changed over the time of shelving ($P < 0.05$). The appearance of rainbow trout did not differ significantly between the two treated groups with WGHE ($P < 0.05$), but the CN + WGHE3 group of the two chitosan and control groups had a significantly inappropriate appearance ($P < 0.05$). For days 3 and 6, the panelists observed no significant difference in fish appearance between groups. On day 6 of the study, the overall acceptability of rainbow trout fillets in C, CN, CN + WGHE1.5, and CN + WGHE3 groups were 2.53 ± 1.06, 2.66 ± 0.89, 3.73 ± 0.96, and 4.33 ± 0.72, respectively. The results showed that the overall acceptability of WGHE treated fish was significantly more than the control group. ($P < 0.05$).

4. Discussion

Many plants are rich sources of polyphenolic compounds, flavonoids, and vitamins or precursors that provide antioxidant and antimicrobial properties, thus providing a proper alternative to antimicrobial compounds and chemical additives (Sharifi-Rad et al., 2015; Shi et al., 2014; Kim et al., 2013). The WGH is considered a waste product with limited use (Oliveira et al., 2008; Fernández-Agulló et al., 2013), but it contains antioxidant compounds of polyphenols and flavonoids and antimicrobials (Rahmani et al., 2018; Akbari et al., 2012; Cosmulescu et al., 2010). Non-edible walnut parts such as green husk, shell, and hull compared to kernel contain much more phenolic and flavonoid compounds and thus can be applied as natural antioxidants in the food industry (Cosmulescu et al., 2013; Akbari et al., 2012). Specifically, the polyphenolic compounds in WGH are much higher than other parts (Ebrahimi et al., 2018; Akbari et al., 2012), and antioxidant compounds' concentration in different parts of walnuts is affected by cultivars (Cosmulescu et al., 2010), genotypes, geographical area, harvest time, and climate conditions, while different conditions have a significant effect on the biosynthesis and accumulation of polyphenolic compounds (Cosmulescu et al., 2014; Amaral et al., 2008; Jakopic et al., 2007). The total phenol content in WGH was at least 32.61, with a maximum of 74.08 GAE mg/g (Akbari et al., 2012). In a study by Akbari et al. (2012), this value of walnut hull was 19.61–36.10 mg/g, while in a study by Cosmulescu et al. (2010), it was significantly lower than the results of the two previous studies with a minimum of 23.84 and a maximum of 46.87 mg/100 g (Cosmulescu et al., 2010). The data available in the literature are consistent with the results of our study, where a 48.3 mg/g gallic acid equivalent was found. These differences are due to various factors that influence the antioxidant activity of WGH, as previously explained. Hence, considering its cost-effectiveness and contents, it can be used as a natural preservative for storing meat products (Salejda et al., 2016).

The total flavonoid content in the walnut pellicle was higher than other parts of the plant and was equivalent to 8.10–14.95 mg/g of catechin (Akbari et al., 2012). On the other hand, in a study by Ghasemi et al. (2011), the lowest and highest flavonoid contents were 3.59 and 22.91 mg/g quercetin of WGH, respectively (Ghasemi et al., 2011)., which is consistent with the result of the flavonoid content of the extract obtained in this study. The differences in flavonoid compounds in the different available studies are subject to similar factors affecting phenolic compounds.

The DPPH assay is usually performed to confirm the activity of free radical scavenging by plants and pure compounds (Pavithra and Vadivukkarasi, 2015; Sousa et al., 2008). DPPH is an organic, stable nitrogen-free radical used to investigate antioxidant capacity widely (Slatnar et al., 2015). As in other studies, WGHE has shown a concentration-dependent scavenging activity (Fernández-Agulló et al., 2013; Kim et al., 2013) and the alcohol-water solvent plays an important role in extracting phenolic compounds from WGH (Fernández-Agulló et al., 2013). Due to antioxidant activity and appropriate capacity of scavenging free radicals by WGHE, the improvement results of this study are consistent with previous studies (Fernández-Agulló et al., 2013; Kim et al., 2013; Akbari et al., 2012).

Rainbow trout is highly susceptible to oxidative spoilage and rancidity due to its high content of long-chain unsaturated fatty acids (Abedi et al., 2016; Hosseini et al., 2016) (LC-PUFA) (Gregory et al.,

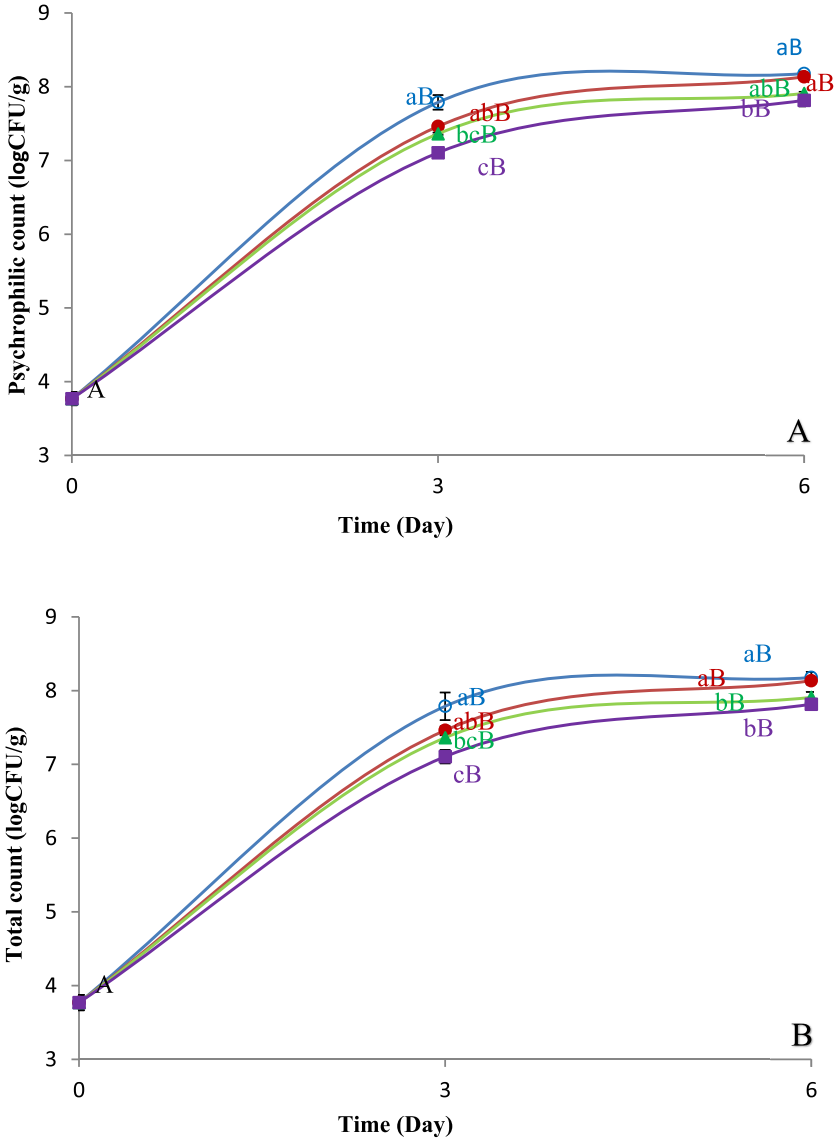


Fig. 5. Psychrophilic count (A) and total count (B) of bacteria in rainbow trout fillet treated with chitosan nanoparticles containing walnut green husk extract (WGHE) over six days storage in the refrigerator (means ± standard errors). ○: Control, ●: chitosan nanoparticles, ▲: chitosan nanoparticles +1.5% WGHE, ■: chitosan nanoparticles +3% WGHE. Different uppercase and lowercase letters represent significant differences between study days and groups, respectively ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2016; Abedi and Sahari, 2014). The appropriate antioxidant properties of the phenolic compounds present in this extract indicate the effect of WGHE to delay the formation of hydroperoxides, which has been confirmed in previous studies (Fernández-Agulló et al., 2013; Kim et al., 2013; Akbari et al., 2012). The significant reduction in TBARS in treated fish fillets with 1.5% and 3% extract indicates effective antioxidant activity on lipid oxidation, which is following the findings of other studies on the effect of natural antioxidants of plants on reducing lipid oxidation and extending the shelf life of rainbow trout (Hassanzadeh et al., 2018; Raeisi et al., 2016; Çoban et al., 2014; Ojagh et al., 2010). Homayonpour et al. (2021) demonstrated that nanochitosan with Cumino cyminum L. EO postponed microbial growth and oxidative processes in sardine fillets by reducing TBAR substances fish has preserved at four °C. Salejda et al. (2016) investigated the positive effects of WGH on the quality of sausages and stated that this cheap herbal compound is a valuable source of functional phytochemicals that could be an excellent alternative to chemical additives. According to the results of our study, the samples treated with nanochitosan exhibited significantly lower oxidizing, indicating the more effectiveness of applying the extract and nanochitosan coating simultaneously. Studies have shown that the antioxidant activity of chitosan and its effect on food shelf life was increased with essential oils (Yuan et al., 2016). This antioxidant capacity results from the presence of chitosan-free amino groups and their reaction with free radicals (Yen et al., 2008). Also, chitosan nanoparticles reduced lipid oxidation and TBA levels in stored silver carp in refrigerators (Ramezani et al., 2015) and freezers (Zarei et al., 2015).

TVB-N increased in all groups due to the nature of the applied treatment to fish fillets, which is associated with the possible breakdown of proteins due to microbial activity and proteolytic enzymes (Tavakoli et al., 2018). Volatile bases, including ammonia, monoethylamine, dimethylamine, and trimethylamine, are results of the catabolism of amino acids in fish tissue, and their accumulation leads to off-odor in fish (Goulas and Kontominas, 2007; Özogul et al., 2004). A significant reduction in the amount of TVB-N compounds in nanochitosan treated groups, especially its combination with WGHE, could be attributed to the reduced microbial count or the reduced capacity of oxidative deamination activity of non-protein nitrogenous compounds by bacteria, or both factors (Fan et al., 2008). The extract of pomegranate skin with chitosan and orange and pomegranate skin in combination with nanochitosan significantly reduced TVB-N in silver carp and rainbow trout fillets, respectively (Berizi et al., 2018; Zarei et al., 2015). The use of natural plant preservatives such as rosemary (Abdollahi et al., 2014),

Table 1
Effect of chitosan nanoparticles containing different concentrations of walnut green husk extract (WGHE) on the sensory properties of rainbow trout fillet over six days of storage in refrigerator.

Storage time (days)	Treatments	Color	Odor	Appearance	Total acceptance
0	C	4.33 ± 0.72 [a]	4.33 ± 0.81 [a]	4.73 ± 0.45 [a]	4.33 ± 0.89 [a]
	CN	4.40 ± 0.82 [a]	4.40 ± 0.82 [a]	4.66 ± 0.48 [a]	4.26 ± 0.88 [a]
	CNWE1.5	4.13 ± 0.99 [a]	4.60 ± 1.05 [a]	4.33 ± 0.61 [ab]	4.06 ± 1.03 [a]
	CNWE3	4.00 ± 1.19 [a]	4.53 ± 0.63 [a]	4.13 ± 0.51 [b]	3.93 ± 1.03 [a]
3	C	4.13 ± 0.99 [a]	3.40 ± 0.82 [a]	4.20 ± 1.03 [a]	2.66 ± 1.29 [a]
	CN	3.93 ± 1.03 [a]	3.46 ± 1.12 [a]	4.33 ± 1.04 [a]	2.53 ± 1.35 [a]
	CNWE1.5	4.20 ± 0.94 [a]	3.86 ± 1.06 [a]	4.13 ± 0.91 [a]	3.06 ± 1.27 [a]
	CNWE3	4.33 ± 1.04 [a]	3.93 ± 1.09 [a]	3.86 ± 1.06 [a]	3.26 ± 1.70 [a]
6	C	3.73 ± 1.27 [a]	2.80 ± 1.26 [a]	3.66 ± 0.89 [a]	2.53 ± 1.06 [a]
	CN	3.80 ± 1.26 [a]	2.66 ± 1.04 [a]	3.86 ± 1.12 [a]	2.66 ± 0.89 [a]
	CNWE1.5	4.00 ± 1.00 [a]	3.26 ± 1.22 [a]	3.93 ± 1.03 [a]	3.73 ± 0.96 [b]
	CNWE3	4.13 ± 1.25 [a]	3.66 ± 1.49 [a]	4.00 ± 1.06 [a]	4.33 ± 0.72 [b]

Likability scores: 1 = poor, 2 = fair, 3 = good, 4 = very good and 5 = excellent. Different letters representing significant difference in different column's ($P < 0.05$). C (control), CN (chitosan nanoparticles), CN + WGHE1.5 (chitosan nanoparticles + 1.5% walnut green husk extract) and CN + WGHE3 (chitosan nanoparticles + 3% walnut green husk extract). Lowercase letters represent significant differences between groups ($p < 0.05$).

cinnamon (Ojagh et al., 2010), and thyme (Goulas and Kontominas, 2007) in combination with chitosan coatings has delayed the production of TVB-N during storage of various fish. In this study, it seems that soluble WGHE and nanochitosan solution have effectively penetrated fish fillets, prevented microbial activities and enzymatic autolysis, and thus reduced the amount of TVB-N lower than the limits (20 N/100 g).

The release of alkaline compounds, including ammonia and biogenic amines due to microbial or endogenous enzymatic activity, leads to an increase in pH values (Chaijan et al., 2005), which results in the change in pH by microbial attack and autolysis (Ghaly et al., 2010). The extracts of various plants such as grape seed (Hassanzadeh et al., 2018) and pomegranate skin (Berizi et al., 2018) along with chitosan, as well as tea extract (Fan et al., 2008) reduced pH during fish storage. The delay in pH increase of the treated samples with WGHE indicates the antimicrobial properties of this extract, and although it has not been able to prevent the overall increase in pH (probably due to high endogenous activities), it showed a positive effect on pH changes compared to the control group. These results clarify the pH and TVB-N co-relation.

Many plant extracts and essential oils have been assessed due to their antimicrobial effects against food-related and food-borne bacteria. For example, Zataria Multiflora oil (Barkhori-Mehni et al., 2017), Pomegranate skin extract (Berizi et al., 2018; Gullon et al., 2016; Kharchoufi et al., 2018), Coconut shell extract (Olatunde et al., 2019; Akinpelu et al., 2015), green Pistachio skin (Rajaei et al., 2010) and WGH (Fernández-Agulló et al., 2013; Oliveira et al., 2008) have good antimicrobial properties. The antimicrobial activity of essential oils and extracts is associated with the components and structural status of the plant oils, their functional groups, probable synergistic activities, and interactions between the associate compounds. The phenolic compounds are the most important components of antimicrobial agents in various species of plants and their extracts or essences (Mahmoodi et al., 2012).

Chitosan also exerts antimicrobial activity on a wide range of microorganisms; however, pure chitosan film did not show important antimicrobial effects against pathogens and spoilage bacteria (Wang and Gao, 2013; Ojagh et al., 2010). Although in our study, chitosan nanoparticles could not impede the growth of psychrophilic bacteria and total microbial community numbers in stored fish fillets in the refrigerator, the combination of WGHE and nanochitosan effectively prevented this increase. The interactions between chitosan and essential oils improve the moisture and surface properties of its coatings, which improve the antimicrobial yield of phytochemicals (Kurek et al., 2014). In the study by Salejda et al. (2016), it was found that WGHE significantly reduced the growth of bacteria and consequently reduced total microbial count in heat-treated sausages (Salejda et al., 2016) The similar results reported by Çoban (2021), revealed that using of chitosan coating with propolis extract could prevent bacterial growth including psychrophilic and mesophilic bacteria and cause an effective improvement in the chemical and organoleptic characteristics of crayfish meat.

Finally, sensory evaluation (Gao et al., 2014) is an effective way to study the quality of food products during shelf life. The higher overall acceptability of the treated groups with the combination of WGHE and CN showed that this extract did not negatively affect the sensory properties of rainbow trout fillet, but it was also favorably evaluated by the panelists. Using chitosan coatings and essential oils to store rainbow trout (Mehdizadeh et al., 2021; Ojagh et al., 2010), silver carp (Abdollahi et al., 2014), Channa Argus (F. Yang et al., 2015), and sardine (Homayonpour et al., 2021) indicated desirable effects on the sensory properties of fish. We conclude that chitosan coatings containing essential oils of plants reduce rainbow trout's chemical and microbial activity during storage, which is significantly and positively associated with food sensory properties (Mehdizadeh et al., 2021; Frangos et al., 2010; Ojagh et al., 2010).

5. Conclusions

Green husk, a byproduct of walnut, is a good source of natural antioxidant and antimicrobial compounds. To the best of our knowledge, alcohol-water extract of WGH was studied for the first time to extend the shelf life of aquatic foods under cold storage. The extract of WGH improved the preservation condition of rainbow trout (*Oncorhynchus mykiss*) by delaying the microbial spoilage and oxidative processes. Combining WGHE with chitosan nanoparticles increased extract efficiency in reducing PV, TBARS, and TVB-N, postponed the pH increase and microbial growth and enhanced the overall end-user acceptability of stored rainbow trout fillets. Considering the reuse of this waste material and according to the results of this study, we suggest the combination of WGHE with CN as a very cost-effective method and an active packaging ingredient to improve the preservation of rainbow trout and other seafood in cold storage.

Declarations of interest

The authors have no conflicts of interest relevant to this article.

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.

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Glossary

WGHE: ethanol-water extract of the walnut green husk
CN: chitosan nanoparticles solution of 2%
C: control
WGHE1.5: 1.5% ethanol-water extract of the walnut green husk
WGHE1.5: 3% ethanol-water extract of the walnut green husk
TVN: total volatile nitrogen
TBARS: thiobarbituric acid reactive substance
PV: peroxide value
LC-PUFAs: long-chain polyunsaturated fatty acids
ω3: omega-3
TPC: total phenolic content
DPPH: 2,2-diphenyl-1-picrylhydrazyl
PCA: plate count agar
FCR: folin-ciocalteu reagent
UV: ultraviolet
MDA: Malondialdehyde
MgO: magnesium oxide
NaCl: Sodium chloride
TVC: total viable count
PVC: psychrophilic viable count
rpm: revolutions per minute
CFU/g: Colony forming unit per gram
SPSS: Statistical Package for the Social Sciences
SD: Standard deviation
DMRT: Duncan's multiple range
ANOVA: analysis of variance
mEq: the milliequivalent is the unit of measure often used for electrolytes
kDa: [kilo- + dalton], A unit of molecular mass consisting of 1000 Da
GAEs: mg of gallic acid equivalents per 100 g of sample
CEs: mg catechin equivalents per 100 g of sample
mmol/L: Millimoles per liter; a unit of measure that shows the concentration of a substance in a specific amount of fluid
mg/mL: milligrams per milliliter
w/v: percent of weight of solution in the total volume of solution
v/v: volume by volume percentage