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Modeling Microbial Population of Coated Sprouted Wheat through Zarrin-Giah Essential Oil in Chitosan Emulsion under Modified Atmosphere Packaging

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Abstract

Due to its health benefits, fresh sprouted cereals are considered popular food source. They are very sensitive and highly susceptible to microbial spoilage during transportation, processing, and storage. This phenomenon makes them potentially high-risk fresh products. This study aimed to assess the effect of emulsion coating consisting of *Dracocephalum kotschyi* essential oil (0, 50, 150, 250, 300 ppm)-chitosan solution (0, 0.3, 0.38, 0.63, 0.75%) during the immersion time (10, 25, 55, 85, 100 s) on the microbial properties of fresh sprouted wheat stored at 4°C. The Response Surface Methodology (RSM) was adopted in modeling the independent variables' effects. The results shown that increase in the essential oil and chitosan solution concentration reduced the microbial spoilage. High concentration of *Dracocephalum kotschyi* oil decreased the fungus population after 12 days. Coating of sprouted wheat at optimized level of independent variables (0.62% chitosan, 57 ppm *Dracocephalum kotschyi* oil and 29.49 s immersion time) reduced the microbial and fungal populations. This treatment can reduce weight loss, and maintain tissue firmness, total phenolic, and ascorbic acid content of the sprouted wheat during cold storage, with no effect on its sensory properties. Our findings indicate that nanoemulsion coating based on chitosan and *Dracocephalum kotschyi* oil at appropriate levels could be beneficial in maintaining sprouted wheat quality and increasing its shelf-life.

Keywords: Badrandjboie-Dennai, Edible coating, Microbiological analysis, Response surface methodology

Abbreviations

RSM	Response Surface Methodology
TPC	Total phenolic content
CCD	Central Composite Design

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Introduction

Food provides components with bioactive properties and various nutrients involved in health improvement and disease prevention (Samtiya *et al*., 2021). Sprouted cereals have been consumed as different types for centuries across the world, especially in Africa and Asia (Marti *et al*., 2018). They are rich sources of proteins, vitamins, minerals and phenols, and bioactive compounds such as glucosinolates, phenolic and selenium all necessary to maintain health (Marton *et al*., 2010). High susceptibility of sprouts to microbial spoilage, due to their high water content (up to 95%) and respiration rate prohibit their long-shelf-life. These features increase the putrefaction and leakage of nutrient rich exudates, tissue damage, and early aging of this product (Turner *et al*., 2020). Different preservation methods like cold storage, chemical immersion, modified atmosphere packaging, and edible coatings are adopted to extend product shelf-life by preventing spoilage, microbial growth and maintaining product freshness. Hurdle technology consists of a combination of preservative methods with synergistic effects on suppressing microbial spoilage, and maintaining nutritional and sensorial properties of perishable products. Combination of edible coating, natural antimicrobial agents like plant essential oil and storage at low temperatures provide the three preventive measures if the shelf-life promotion of the fresh products is sought (Moradi *et al*., 2019; Yavari & Abbasi, 2022).

The edible coating is a thin-layer packaging material consisting of lipids, polysaccharides, proteins, or their combination. Because edible coatings limit gas permeability, they can prevent the oxidative reactions, texture softening, water loss, and microorganisms' proliferation (Benhabiles *et al*., 2013; Eyiz *et al*., 2020). Chitosan is a polysaccharide, obtained by alkaline deacetylation of chitin, with high potential of application as a biodegradable edible coating or film in food packaging (Zhu *et al*., 2008; Saki *et al*., 2019). The degree of deacetylation and the molecular weight of chitosan are highly effective factors on its physicochemical properties, quality, and application. Chitosan-based films and coatings have multi-function positive effects on products (Sridhar *et al*., 2021). Chitosan based edible coating of fresh fruits increase postharvest shelf-life and reduce quality deterioration (Benhabiles *et al*., 2013). Chitosan-based coatings decrease water loss, respiration rate, and microbial contamination of fresh fruits. It has the same effect as modified atmosphere storage in changing the internal gas composition (Petriccione *et al*., 2015).

Incorporating herbal essential oil into edible coatings improves their mechanical, functional, organoleptic, and nutritional features (Rastegar & Atrash, 2021) Essential oils are volatile oily liquid obtained from different plants and applied as food flavorant, antioxidant, antifungal, antiviral, or insecticidal agent (Saki *et al*., 2019; Eftekhari *et al*., 2021). *Dracocephalum* is a genus of flowering plants in the Lamiaceae family with proper source of flavonoids, terpenoids and alkaloids like luteolin, apigenin, oleanolic acid, ursolic acid, geranial, neral, limonene-10-al and rosmarinic acid. *Dracocephalum kotschyi* (locally known as Zarrin-giah or Badrandjboie-Dennaie) contains valuable essential oil that is enriched in different compounds like citral, caryophyllene, terpinyl, acetate, limonene, α-terpineol, δ-3-carene, α-pinene, terpinen-4-ol, geranial, limonene-10-al, 1,1-dimethoxydecane, Gerania, α-pinene), (Heydari *et al*., 2019; Khodaei *et al*., 2018). Phenolic compounds like caffeic acid, chlorogenic acid, phenylpropanoids, and flavonoids in *Dracocephalum* genus contribute to antioxidant and antimicrobial activities. Consumption of *Dracocephalum Kotschyi* essential oil is considered safe (GRAS) for human by the Food and Drug Administration (Heydari *et al*., 2019; Khodaei *et al*., 2018). Food deterioration through spoilage microorganisms during storage has a major

impact on the physicochemical and qualitative properties, and also shelf life of perishables fresh agricultural products (Enyiukwu *et al*., 2020).

The object of this study is to assess a combined preventive approach of chitosan coating incorporated with *Dracocephalum kotschyi* oil as a natural antimicrobial substance to control the microbiological quality and extend the shelf-life of sprouted wheat during cold storage. Response surface methodology is adopted in modeling the changes in microbial population and obtains the best consumption levels of *Dracocephalum kotschyi* essential oil, chitosan and soaking time. The qualitative properties of the treated sample at the best conditions was compared to the control sample.

Materials and methods

Materials

Dracocephalum kotschyi was purchased from Research Center for Medicinal Plant Resources, Isfahan, Iran. The *Dracocephalum kotschyi* essential oil was extracted by steam distillation in Clevenger apparatus (AVZH-CLNGR, Pioneers of Iranian Nanomaterials, IRAN) and dried over anhydrous sodium sulfate and refrigerated at 4 ℃ in dark glass containers. The quantitative and qualitative analyses of *Dracocephalum kotschyi* oil was run by applying gas chromatography coupled to mass spectrometry (GC/MS), (Agilent 6890N, USA), (Martucci *et al*., 2015). The primary components of this oil consist of Perillaldehyde (18.53%), Carvacrol (12.99%), α-Pinene (10.42%), Eugenol (10.11%), E-β Damascenone (8.64%), Geraniol (7.82%), Limonene (7.63%) and Terpinen (5.78%).

The Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-dichloroindophenol, sodium carbonate, trichloroacetic acid and methanol were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Other chemicals were purchased from Merck Co. (Darmstadt, Germany).

Sprouting of wheat seeds and assessment of their qualitative properties

The sprouting process follows the method described by Khaskheli *et al*. (2019), with slight modifications. First, the seeds were soaked for 10 min in a 0.07% sodium hypochlorite sanitizers solution, and were then immersed in distilled water for 6 h. Water was then removed and the seeds were spread on moist filter paper, and finally they were incubated in a growth chamber (Teifazma, TAT-J55, Iran) at 29 ± 2 °C and 85% humidity for 72 h.

The moisture, protein, lipid, carbohydrate, crude fiber, and ash content of sprouted wheat were measured in accordance to the AOAC 945.38 standard method.

The Mg, Mn, K, Ca, Mg, Fe, Zn content of sprouts were determined by applying the atomic spectrometry (Shimadzu AA-6200, Japan).

Preparation of *Dracocephalum kotschyi* oil-chitosan nanoemulsions

To prepare chitosan dispersion, it was weighted (Zurich 4000C, Switzerland) according to Table 1 and added to a beaker containing acetic acid (0.5 %v/v). The solution was heated to 45 °C, mixed on a magnetic stirrer (Heidolph-MR 3001, Germany) at 10,000 rpm for 10 min to complete dissolution and the pH was adjusted to 5.2 with NaOH (Petriccione *et al*., 2015; Maleki *et al*., 2018). The *Dracocephalum kotschyi* oil (0-300ppm), as the oil phase, the Tween 80 (100 ppm) and chitosan dispersions (0-0.75% w/w) in deionized water as the aqueous phase were subjected to sonicatation at 20 kHz (Fisher Scientific, 705 Sonic Dismembrator, USA) to form nanoemulsions (Rashid *et al*., 2020; Kotta *et al*., 2015).

Treating sprouted wheat by applying *Dracocephalum kotschyi* oil-chitosan nanoemulsions

Sprouted wheat was immersed in the *Dracocephalum kotschyi* oil-chitosan nanoemulsions and then dried at 25 ± 1 °C. The samples were packed under modified atmosphere (30% O_2 and 70% N_2) in polyethylene bags, and stored at 4 ±1 °C, 80-

85% relative humidity for 12 days (Maleki *et al*., 2018; Moradi *et al*., 2019).

Microbiological analysis

Total microbial count was determined by applying the tablet colony counting method (standard plate count) according to Yavari & Abbasi (2020). Briefly, 10 g of the sample was immersed in 90 ml saline solution and vortexed (Seward Medical, London, U.K.) for 2 min. The samples were analyzed for total microbial counts by incubation (B Series Incubator, BINDER Inc., USA) at 37 °C for 48 h on plate count agar (PCA). Data was expressed as log colony forming units (CFU)/g sample.

The total yeast and mold count were determined according to Maleki *et al*. procedure. A 20 g sample and 180 ml of 0.1% peptone water were homogenized into the stomacher bags (Seward Medical, London, U.K.) for 1 min. At this stage, the serial decimal dilutions were prepared, and 100 µl of the diluted sample was spread on potato dextrose agar (PDA), and plates were incubated at 25 °C for 5 days.

Experimental design

The central composite design (CCD) in Response Surface Methodology (RSM) was applied to assess the independent variables' effects [chitosan solution concentration 0-0.75% (X_1) and *Dracocephalum kotschyi* oil concentration 0-300 ppm (X_2) in *Dracocephalum kotschyi* oil-chitosan nanoemulsions and immersion time 10-120 s (X_3)] and their interactions on responses. 20 treatments with six central points as shown in Table 1 were developed. The independent variables' function in a second-order polynomial equation is expressed as follows:

Table 1- Effect of independent variables on microbiological qualities **of wheat sprouts**

Run	Variables			Response			
	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4
1	0.13	50	25	10.22	7.86	11.91	5.70
2	0.13	250	25	10.08	7.77	11.78	0.00
3	0.63	50	25	9.67	7.30	11.51	6.48
4	0.63	250	25	10.02	7.65	11.83	6.40
5	0.13	50	85	10.20	7.77	11.96	6.30
6	0.13	250	85	10.10	7.80	11.90	6.30
7	0.63	50	85	9.70	7.39	11.35	0.00
8	0.63	250	85	10.23	7.93	11.93	6.78
9	0.38	0	55	10.08	7.71	11.81	0.97
10	0.38	300	55	8.30	7.08	11.26	6.30
11	0.00	150	55	10.04	7.80	11.90	6.30
12	0.75	150	55	9.47	7.32	11.29	6.00
13	0.38	150	10	9.87	7.45	11.66	6.60
14	0.38	150	100	10.04	7.78	11.88	6.48
15	0.38	150	55	9.93	7.58	11.70	5.70
16	0.38	150	55	9.90	7.69	11.71	6.00
17	0.38	150	55	9.92	7.66	11.59	5.70
18	0.38	150	55	9.81	7.66	11.65	6.18
19	0.38	150	55	10.01	7.71	11.54	6.18
20	0.38	150	55	10.04	7.55	11.64	5.70

X_1: chitosan solution concentration (%), X_2: *Dracocephalum kotschyi* oil concentration in *Dracocephalum kotschyi* oil-chitosan nanoemulsions (ppm), X_3: immersion time (s), Y_1: microbial count, Y_2: total yeast and mold count, Y_3: microbial count after 12 days, Y_4: total yeast and mold count after 12 days.

$$Y=a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 \quad (1)$$

where a_0 is the constant, a_1, a_2 and a_3 are the linear, a_{11}, a_{22} and a_{33} are the quadratic and a_{12}, a_{13} and a_{23} are interactive coefficients. The coefficients of the response surface equation were determined in Design-Expert 7.1.1 software environment (Rezvain *et al.*, 2020; Mazrouei Sebdani & Abbasi, 2023).

Comparison of qualitative features of optimal and control samples

After modeling and optimizing the independent variables' effect on dependent variables change, the optimum and control samples were assessed according to the instructions for quality characteristics such as pH, weight loss (%), ascorbic acid (mg/100g), total phenolic compounds (mg GAE/100g), firmness (N), and total microbial count (log CFU/g) at intervals 0, 4, 8, 12, 16 and 20 days. The sensory assessment was evaluated immediately after production.

pH

The samples' pH was assessed according to Maleki *et al.* (2018) procedure through a pH meter (Metrohm Ltd. Herisau, Switzerland).

Weight loss

The samples' weight loss was determined by weighing the samples on the initial day and after storage time by a digital balance (Zurich 4000C, Switzerland). Weight loss was determined through Eq. (2) (Maleki *et al.*, 2018; Rastegar & Atrash, 2021):

$$\text{Weight loss } (\%)=(W_a-W_b/W_a)100 \quad (2)$$

where W_a is the sample weight on the first day, and W_b is the weight after storage time.

Firmness

The sprouts' firmness was determined by the texture analyzer (Brookfield AMETEK CT3-115 LFRA, USA) equipped with a 39 mm cylindrical probe. The samples were penetrated for 40 mm at a 60 mm s^{-1} speed, and the force volumes were expressed as newton (N), (Maleki *et al.*, 2018; Moradi *et al.*, 2019; Eyiz *et al.*, 2020).

Ascorbic acid

The level of ascorbic acid in the samples was determined based on the titrimetric method by 2,6-dichlorophenolindophenol. First 10g of the sample was blended with 50 ml of 5% w/v trichloroacetic acid, the mixture was then poured into a 100 ml volumetric flask, shaken, filtered, made up to volume and then, 10 mL of solution was titrated against 2,6-dichloroindophenol until the solution color changes into pink for 15 s. The Ascorbic acid content was expressed as mg/100g weight basis of sample (Huang *et al.*, 2017; Eyiz *et al.*, 2020).

Total phenolic content (TPC)

The extraction of phenolic compounds was done according to Rastegar & Atrash (2021) procedure. To prepare the methanol extract, 5.0 g of sprouted sample was homogenized by 15 ml of methanol (80%) and centrifuged (10,000×g) for 15 min. The supernatant was applied in analyzing total phenolic content and antioxidant activity. The TPC was determined according to the colorimetric Folin–Ciocalteau method. First, 0.125 ml of the methanol extract was mixed with 1.5 ml Folin–Ciocalteu reagent (1:10 diluted), and after 5-6 min, 1.25 ml of Na_2CO_3 (7.5% w/v) was added and the mixture was kept at room temperature for 60 min. The absorbance was recorded at 760 nm by UV–Vis spectrophotometer (UV-1800, Shimadzu, Japan). The results were represented as mg gallic acid equivalent (mg 100 g–1of the dry weight), (Nouri & Abbasi, 2018).

Statistical analysis

The obtained data were analyzed in a completely randomized design through SPSS 12.0 (SPSS Inc., Chicago, IL, USA). The means were compared by applying LSD at $p < 0.05$ level. Statistical analysis of sensorial properties was run through Kruskal–Wallis test. All tests were performed at least in three repetitions and Values are presented as means ± SD

Results and Discussion

Proximate compositions and functional compounds of wheat sprouts

Seed germination is a proper process that affects composition and bioavailability of the seeds' content. The concentration of compounds in sprouted wheat are tabulated in Table 2. The high moisture content in the product reduces shelf life and increases spoilage probability. This product is an important resource of protein, dietary fiber, and minerals like potassium, calcium, magnesium, manganese and iron.

The results indicated that the sprouted wheat is a proper source in providing nutritional requirements of human body. According to available records, germination is known as a proper process affecting the composition and bioavailability of seeds components. The sprouted wheat is introduced as a rich source of protein (13.24%), fat (3.89%), total phenolic (3.2 mg GAE/g), minerals and vitamins (Ghavam *et al*., 2021). Sprouted grains and beans like black mung bean are a great source of phenolic acids, antioxidants like vitamins C and E, beta-carotene and flavonoids, including hydroxybenzoic acids, hydroxycinnamic acids and C-glycosidic (Feng *et al*., 2018).

Table 2- Proximate compositions of wheat sprouts

Components	Value
Moisture (%)	47.31±1.2
Protein (%)	11.69±0.93
Lipid (%)	2.50±0.70
Crude fiber (%)	3.49±1.80
Ash (%)	0.50±0.50
Ascorbic acid (mg/100g)	1.72±0.09
Na (mg/kg)	19.99±0.04
K (mg/kg)	6993±0.04
Ca (mg/kg)	79.13±0.90
Mg (mg/kg)	129.40±0.03
Mn (mg/kg)	10.98±1.10
Fe (mg/kg)	10.30±0.22
Zn (mg/kg)	8.92±0.06

All values represent the mean of three replicates expressed as mean±SD.

Microbial analysis

Fresh fruit and vegetables' deterioration is due to spoilage caused by microorganisms. The effect of *Dracocephalum kotschyi* essential oil, chitosan concentrations, and soaking time on total microorganism and yeast and mold count of sprouted wheat after production and at the 12th storage day was assessed. Quadratic models were developed to describe the independent variables' influence on microbial analysis. Soaking Time had no significant effect on total microorganism count. The linear terms of chitosan and *Dracocephalum kotschyi* oil concentration had significant effect on bacterial count after production and at the 12^{th} storage day ($P< 0.0001$). A remarkable decrease was observed in the bacterial population after production at the highest concentration of chitosan, Table 3. The Interaction of independent variables had synergistic effect on the bacterial count reduction, Fig. (1A-C). The contribution of *Dracocephalum kotschyi* essential oil on inhibiting the microorganism growth is due to the existence of compounds like perillaldehyde, carvacrol, limonene, E-β Damascenone, and geraniol with antibacterial properties (Ghavam *et al*., 2021). Ghavam *et al*. found considerable antimicrobial effect of *Dracocephalum kotschyi* oil on different microorganisms like *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Aspergillus niger*, *Shigella dysenteriae*, *Salmonella typhi* and *Pseudomonas aeruginosa*. The antimicrobial action of chitosan is associated with the formation of a

polyelectrolyte composition, because its protonated amine groups selectively bind to the microorganisms' negatively charged cell surface, cause the loss of components and inhibit microbial growth (Pizato *et al*., 2022). Chitosan films with 20% carvacrol (the main terpene of oregano essential oil) reduced the *Pseudomonas fragi*, *Shewanella putrefasciens*, and *Aeromonas hydrophila* growth (Gutierrez-Pacheco *et al*., 2020). The antibacterial activity of chitosan films against *Staphylococcus aureus* and *E. coli* increased after adding 10 $mg \cdot mL^{-1}$ carvacrol (Gutierrez-Pacheco et al., 2020). Chitosan film incorporated with *Thymus piperella* essential oil reduced the *Serratia marcescens* and *Listeria innocua* growth (Gutierrez-Pacheco *et al*., 2020). The coating nanoparticle of chitosan on fresh-cut apples acts as a barrier to moisture and a well-dispersed coating with a greater antimicrobial impact on microorganisms (Pilon *et al*., 2015).

Table 3- Regression coefficients of predicted polynomial models for assessing responses

Coefficient	Responses			
	Y_1	Y_2	Y_3	Y_4
X_0	10.50^{***}	8.00^{***}	12.33^{***}	6.00^{***}
X_1	-0.57^{**}	-1.24^{**}	-1.41^{*}	2.90^{ns}
X_2	$-5.48\times10^{-3***}$	-3.35×10^{-3ns}	$-4.87\times10^{-3**}$	-4.28×10^{-3ns}
X_3	–	$+2.13\times10^{-3*}$	–	-0.042^{ns}
X_{12}	$+5.64\times10^{-3**}$	$+4.75\times10^{-3**}$	$+5.51\times10^{-3**}$	$+0.061^{***}$
X_{13}	–	–	–	$-5.23\times10^{-4***}$
X_{23}	–	–	–	-0.21^{***}
X_{11}	-1.13^{**}	–	–	–
X_{22}	$+1.42\times10^{-5**}$	$+8.39\times10^{-6*}$	$+1.23\times10^{-5**}$	$-1.80\times10^{-4***}$
X_{33}	–	–	–	$+4.23\times10^{-4*}$
Lack of fit	0.487^{ns}	0.204^{ns}	0.126^{ns}	0.127^{ns}
R^2	0.86	0.85	0.81	0.88
CV (%)	0.84	1.06	0.81	7.88

CV: Coefficient of variation
Ns: Not significant ($p > 0.05$).
*: Significant at $p \leq 0.05$.
**: Significant at $p \leq 0.01$.
***: Significant at $p \leq 0.001$.

X_1: Chitosan concentration (%), X_2: *Dracocephalum kotschyi* oil concentration in chitosan (ppm), X_3: Immersion time (s)

Y_1: Microbial count, Y_2: Total yeast and mold count, Y_3: Microbial count after 12 days, Y_4: Total yeast and mold count after 12 days

The sprouted wheats' fungus count was reduced after production by increasing chitosan concentration. A considerable reduction in fungus population was observed at the 12th storage day at highest *Dracocephalum kotschyi* essential oil concentration, Table 3. The variables' interaction at the middle levels had reducing effect on product fungus immediately after production, Fig. (1B). At the highest levels of chitosan concentration and immersion time, fungus population decreased at the 12th storage day, Fig. (1E). The interaction effect of *Dracocephalum kotschyi* oil concentrations with chitosan and immersion time was proper in reducing the fungus population at the 12th storage day, Fig. (1D, F). Edible coatings or films with essential oils can increase the product shelf-life and control the microbial quality of fruits and vegetables. Chitosan with lemon essential oil had a positive effect in reducing the fungal growth on strawberries stored at 5ºC (Perdones *et al*., 2012). The application of pectin with lemon essential oil was efficient in reducing the growth of molds and yeasts in strawberries stored under refrigeration (Pizato *et al*., 2022). Chitosan with and without oregano essential oil had fungicidal activity against *Botrytis cinerea*, *Penicillium sp*., *Rhizopus stolonifer*, and *Alternaria alternata*.

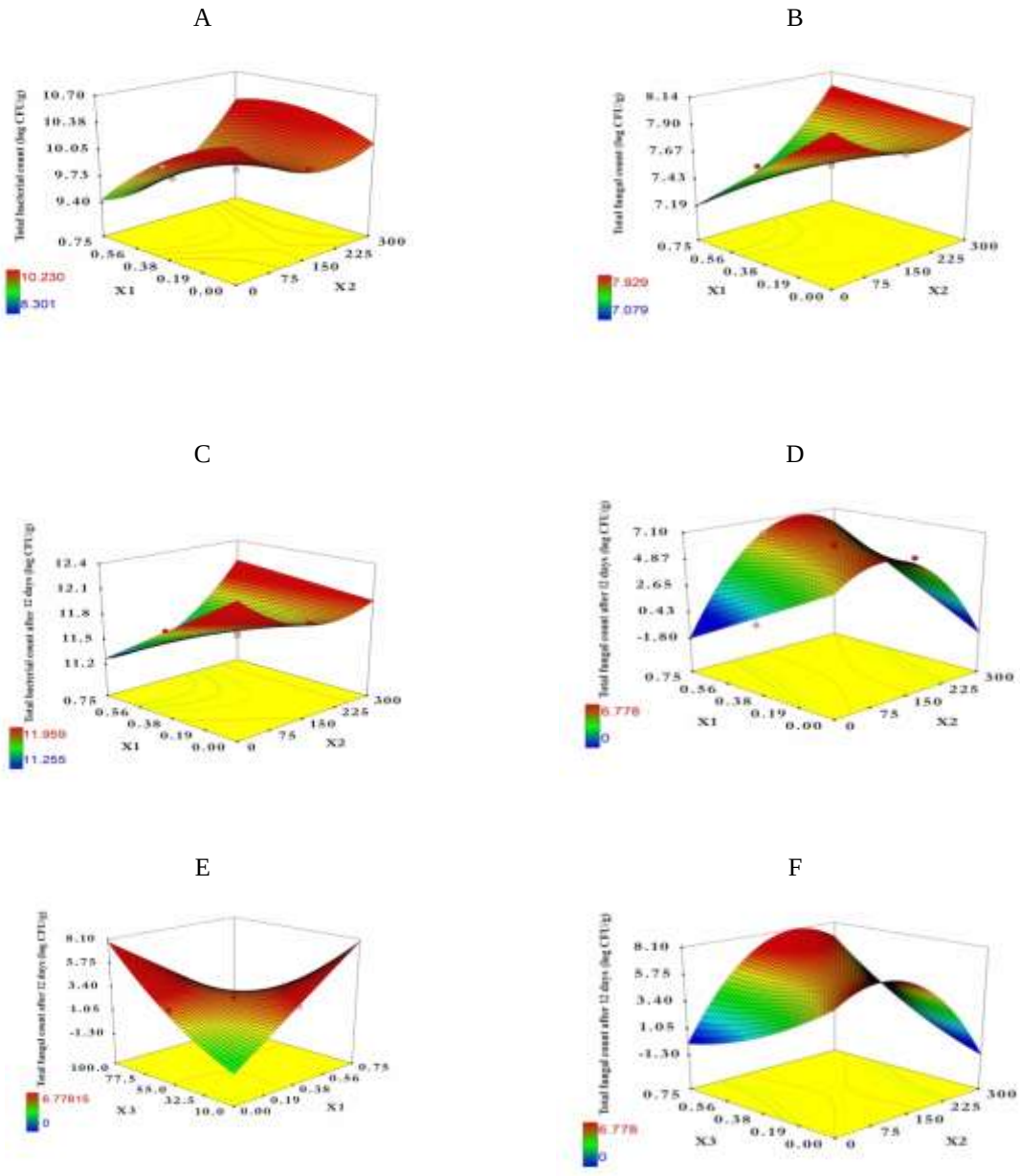

Fig. 1. Response surface plots for the interaction effects of independent variables on the microbial changes of wheat **sprouts**

X_1: Chitosan concentration (%), X_2: *Dracocephalum kotschyi* oil concentration in chitosan (ppm), X_3: Immersion time (s)

Inhibitory effect of chitosan coating on fungal growth follows two mechanisms: 1) chitosan may induce the synthesis of chitinase in plant tissues, which degrades microbial cell walls, and 2) polycationic nature of chitosan may scavenge the major anionic species on the surface of fruits that alter the membrane cell permeability and disorganize molecule with morphological and structural changes in fungal cells (Maleki *et al.*, 2018; Gutierrez-Pacheco et al., 2020).

Molds have an absolute requirement for O_2, and packaging in low O_2 and high CO_2 condition extremely control the fungal growth.

Therefore, the atmospheric change from aerobic to anaerobic situation in coating is not proper for molds and yeasts activity (Maleki *et al*., 2018; Thirupathi Vasuki *et al*., 2023).

The antimicrobial activity of *Dracocephalum kotschyi* oil is due to the effect of different compounds like limonene in disrupting the cell membrane complex of microorganisms (Ghavam *et al*., 2021), and reduce of O_2 diffusion through coating and allows a higher CO_2 concentration surround the products mainly because of the resistance to gas diffusion as a result of the lipophilic nature of *Dracocephalum kotschyi* oil (Moradi *et al*., 2019; Mohammadi *et al*., 2021).

Optimization of sprouted wheat coating through *Dracocephalum kotschyi* oil-chitosan emulsions

The objective of this study was to determine the optimal values of the independent variables in sprouted wheat treatment to improve microbial quality. After modeling, the numerical optimization method was applied to achieve the optimum conditions (minimum microbial and fungal counts) of the product. Coating wheat sprouts with nanoemulsion containing 57 ppm *Dracocephalum kotschyi* oil in 0.62% chitosan solution with 29.49 s immersion time provided the best condition for treating this product and controlling the microbial activity. The predicted results correspond to the experimental results obtained at laboratory validated the RSM models are shown in the Table 4.

Table 4- Validation of RSM models

Responses	Predicted value	Experimental value	Error percentage
Total bacterial count after production (log CFU/g)	9.63	9.54	0.94
Population of fungus after production (log CFU/g)	7.30	7.15	2.09
Total bacterial count at the 12th storage day (log CFU/g)	11.40	11.41	0.08
Population of fungus at the 12th storage day (log CFU/g)	5.69	5.18	9.84

Comparison of qualitative and microbial characteristics of coated wheat sprouts and control during storage

The best level of independent variables was selected to control the sprouted wheat microbial quality. The effect of treating wheat sprouts at optimal conditions during the storage time was assessed on the quality properties like the pH, weight loss, ascorbic acid, total phenol contents, firmness, and total microbial and fungal count of the product.

The coated wheat sprouts had lower pH than the uncoated sample at the beginning of production. During storage, the control pH decreased until the 8th day and then increased. This phenomenon is due to the sprouts spoilage and formation of alkaline autolysis compounds and fungal metabolites (Huang *et al*., 2017; Maleki *et al*., 2018). Changes in the coated sample pH in the storage period were lower than that of the control sample, therefore, the pH of the coated sample was lower than that of the control at the 12th, 16th and 20th of the storage days, Fig. (2A). Changes in pH are associated with 1) the effect of treatments on the respiration rate and 2) metabolic activity that change the biochemical components of the products (Maleki *et al*., 2018). The difference in the loss of water during storage time is other reason of changes in pH (Benhabiles *et al*., 2013). The pH changes of Chinese kiwi fruit have been attributed to the respiration consumption in living cells and oxidation that are responsible of organic acids reduction (Huang *et al*., 2017).

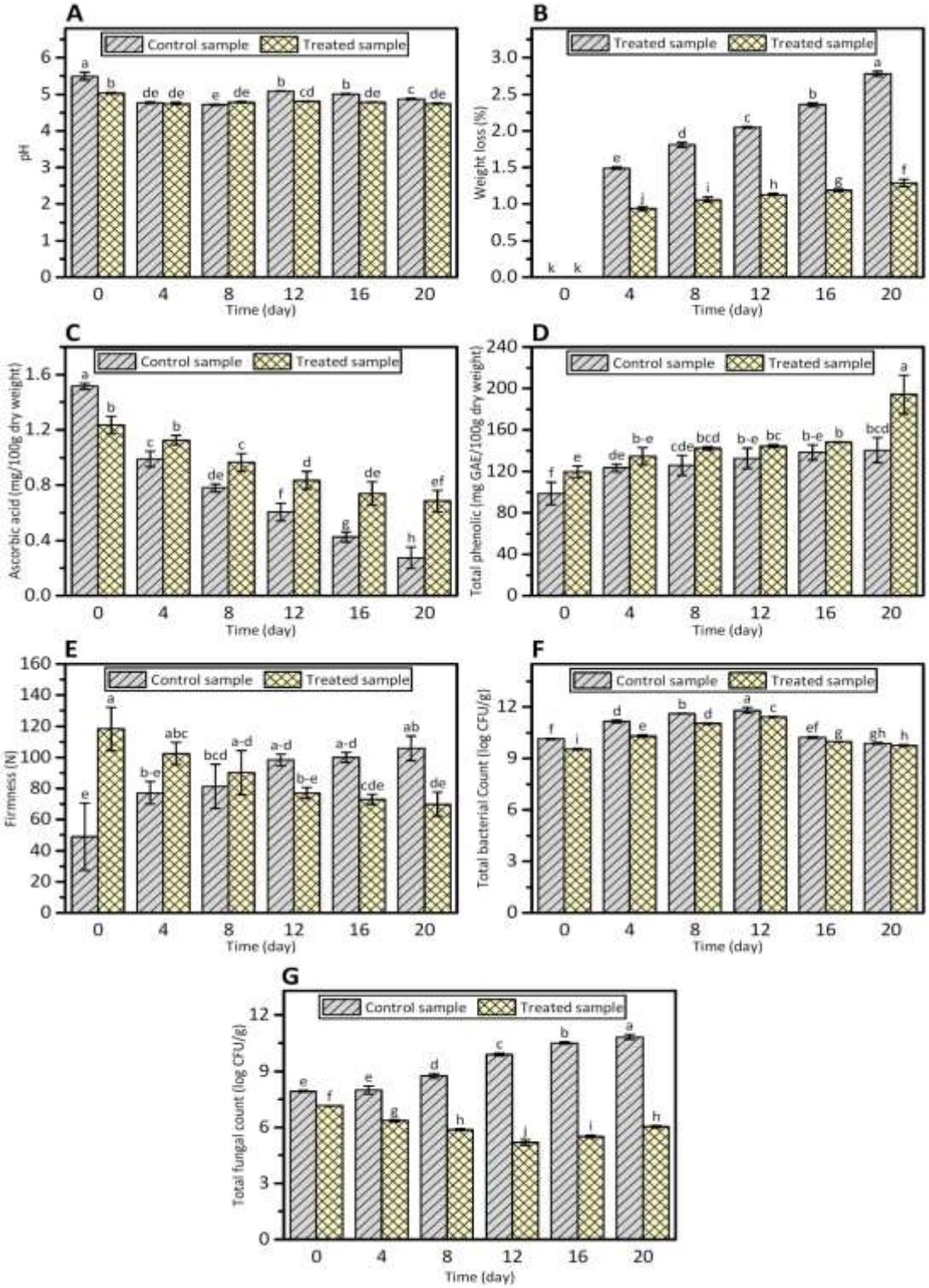

Fig. 2. The qualitative and microbial properties of the coated and uncoated sprouted wheat during storage at 4 ± 1 °C

Product weight loss is one of the most important factors limiting the fresh vegetables and fruits' shelf-life in storage period (Huang *et al*., 2017; Maleki *et al*., 2018). The weight loss of coated sample after 4th, 8th, 16th and 20th days, was lower than the uncoated product, Fig. (2B).

At the last days of product storage (days 12th, 16th, 20th), the weight loss in both the treated and control samples increased considerably. This phenomenon is attributed to transpiration and respiration of product, due to a sharp increase in microbial and fungal growth. Chitosan-based coating reduced respiration and oxidation reactions during storage period by hindering water and gas exchange (Zhu *et al*., 2008). The *Dracocephalum kotschyi* oil incorporated into the chitosan coating as a polysaccharide-based edible coating formed a barrier layer on the surface of the sprouts that reduced the sprouts surface evaporation and respiration rate (Moradi *et al*., 2019). Incorporating of Mentha spicata essential oil as an antioxidant agent into coating of strawberries reduced O_2 diffusion, diminished the oxidative stress, decreased the rate of water loss and consequently reduced fruit senescence (Ghavam *et al*., 2021).

The ascorbic acid content of uncoated sample decreased during storage, while, in the coated sample, did not change until the 4th day.However, ascorbic acid content decreased on 8th, 12th, 16th and 20th storage days. Spontaneous oxidation in the presence of oxygen is the main reason of ascorbic acid deterioration during storage, which was reduced in the treated sample (Moradi *et al*., 2019). Change in the ascorbic acid content in the coated sample was lower than that of the control sample Fig. (2C). Coating materials form a protective membrane between sprouted wheat and its surrounding atmosphere which decrease moisture transfer, O2, and CO2 exchange, and finally prevents the ascorbic acid oxidation. *Dracocephalum kotschyi* oil in chitosan coatings increased their antioxidant activity thus, maintaining the ascorbic acid in the coated product during storage (Moradi *et al*., 2019). Toğrul & Arslan (2004) found that coating tangerines with Carboxymethyl cellulose prevented ascorbic acid loss during storage. Oms-Oliu *et al*. coated pear slices with alginate-containing films and reported that the ascorbic acid content and antioxidant activity remained unchanged at the end of storage period. Bilbao-Sainz *et al*. reported that a layer-by-layer alginate and fungal chitosan based edible coating preserves the ascorbic acid content in fruit bars.

During cold storage, TPC of coated samples was higher than that in the uncoated one. TPC of the coated and uncoated samples in the 20th day of storage followed an ascending trend, Fig. (2D). Edible coatings are effective in reducing oxygen supply for enzymatic oxidation of phenolic compounds by providing a barrier layer to gas exchange on product surface (Moradi *et al*., 2019). *Dracocephalum kotschyi* oil-chitosan emulsion provides a semi-permeable barrier for gas exchange, which lowers the reduction rate in metabolism, oxidative and browning reactions. Preservation of phenolic compounds in sprouts treated with chitosan coating at the end of storage was promoted by reducing polyphenol oxidase activity and actualizing the phenylalanine ammonia lyase (PAL) (Moradi *et al*., 2019). Chitosan, arabic gum and alginate coatings had a positive effect on preserving total phenolics of carambola fruit during storage (Gol *et al*., 2015).

There was a significant difference in firmness of coated and uncoated samples. Firmness in coated sample was higher than the uncoated sample after production and at 4th day ($P< 0.05$). The coating material formed a compact structure and increased the product hardness (Bibao-Sainz *et al*., 2018). At the end of the storage period, firmness of the coated sample was almost similar to the uncoated one upon production. Firmness of the coated sample is due to the activity of texture hydrolyzing enzymes and respiration decreased on 12th, 16th and 20th day, Fig. (2E), while, the firmness of the uncoated sample increased on 8th, 12th, 16th and 20th of storage days. This phenomenon is attributed to more water evaporation and weight loss impact from sprouted wheat surface on increasing firmness. Textural properties of products are one of the primary criterions that limit the shelf-life of fruits and vegetables which are affected by different factors. Water evaporation and weight loss are the major

reason of increasing product firmness. Appropriate coatings with control weight loss can reduce the firmness of product more than control in storage period (Rastegar & Atrash, 2021). Softening is mainly due to cell structure deterioration by pectin hydrolysis in the cortical parenchyma cell wall and middle lamella of fruits and vegetables, This process involves depolymerization and decomposition of the chain length of pectin substances and leads to an increase in pectinesterase and polygalacturonase activities (Moradi *et al*., 2019). Microorganisms' activity is the other factor affecting on the texture changes of agriculture products. Edible coatings based on protein and polysaccharide can limit gas exchanges on fruit and vegetable surface causing a rise in carbon dioxide and a fall in oxygen concentrations that reduce pectinesterase and polygalacturonase activities in the texture of the coated products (Moradi *et al*., 2019). Antimicrobial compounds can inhibit microorganisms' growth and prevent the texture decomposition in fresh product. Carvacrol, limonene, eugenol, geraniol, E-β damascenone, and α-pinene in *Dracocephalum kotschyi* oil have unique antimicrobial properties. The impact of these factors causes texture change in the fresh product during storage.

Edible sprouted wheat has an ample surface area with high moisture content and nutrients, forming a proper environment for microorganisms' growth (Moradi *et al*., 2019). Microbial population, especially fungus increased during storage period, while, the coated sample had lower bacterial and fungus than uncoated Fig. (2 F-G). *Dracocephalum kotschyi* oil and chitosan suppressed microbial spoilage in sprouted wheat not only with their antimicrobial properties but their promotion of decay resistance through preserving the phenolic compounds and ascorbic acid (Saki *et al*., 2019). Applying different types of essential oil can be recommended as a safe method for extending shelf life of fruits and vegetables by controlling fungal decay without any harmful effects on products (Jhalegar *et al*., 2015). In this context, Chitosan-based edible coating containing 0.6% cinnamon inhibit the microorganism growth on fresh-cut potatoes (Wang *et al*., 2011).

In the sensory assessment, no considerable difference was observed between coated and uncoated samples as to odor, flavor, color, texture and overall acceptance ($P > 0.05$), Table 5. The results revealed beneficial effects of this treatment in terms of delay in microbial spoilage without significant changes in the organoleptic properties of the product. In the other study, Guerra *et al*. found that the coated cherry tomatoes with chitosan and peppermint oil had not significant difference in sensorial scores than the control after 24 days of storage. Combination of chitosan and thymol had a positive influence on sensorial properties of fresh fig (*Ficus carica* L.) fruit during 20 days storage at 6 °C (Saki *et al*., 2019). The color of strawberry did not change significantly by coatings with *Aloe vera* gel enriched with basil (*Ocimum basilicum* L.) essential oil, and the glossy surface was the main reason for the high acceptance of the coated fruit (Mohammadi *et al*., 2021).

Conclusion

The *Dracocephalum kotschyi* oil-chitosan nanoemulsions have a positive effect on wheat sprouts' quality by reducing bacterial and fungal growth. The effects of variables' interactions on product quality protection are higher than their independent effects.

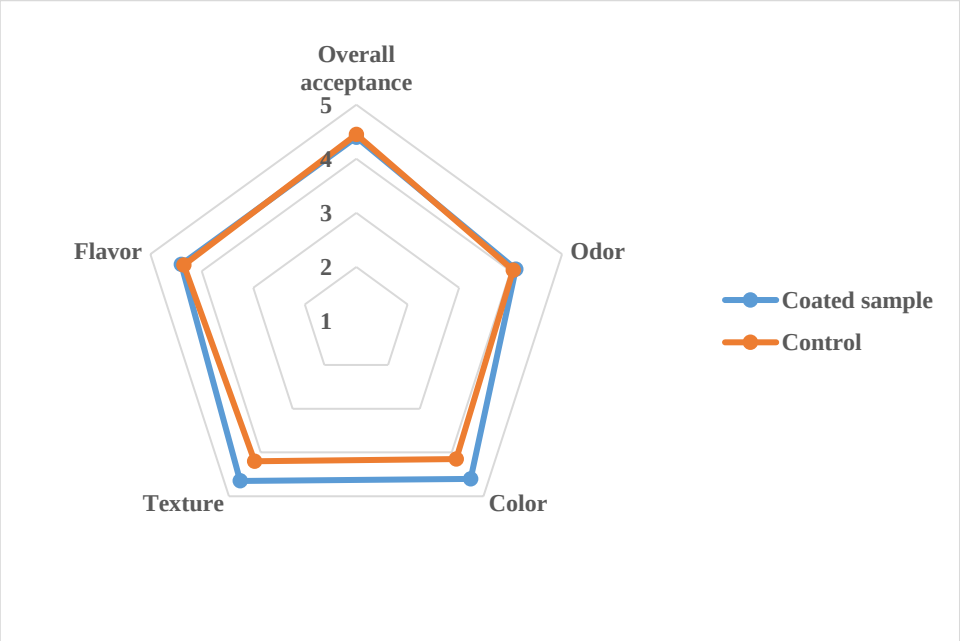

Fig. 3. Effect of edible coating based on chitosan and *Dracocephalum kotschyi* oil on flavor, odor, color, texture and overall acceptability of sprouted wheat stored at 4 °C after 20 days

The best edible coating is formulated at 0.62% chitosan, 57 ppm *Dracocephalum kotschyi* oil and 29.49 s immersion time which extend the product shelf-life. The TPC and ascorbic acid contents of coated sample are higher than that of the control. The treated sprouted wheat has significant low weight loss, and microbial populations than the uncoated sample during storage time. The sensory evaluation of the coated sample received the highest score for overall quality. Application of this emulsion is promising in preserving the safety and quality of sprouted wheat as a valuable and perishable product during cold storage. Despite considerable findings of this article, further studies on combination of new treatments are required to preserve quality of sprouted cereal.

Declarations

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Data availability: The datasets generated and/or analyzed in this study are available from the corresponding author upon request.

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Informed consent: Not applicable

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مدلسازی جمعیت میکروبی جوانه گندم پوشش داده شده با امولسیون حاوی اسانس زرین‌گیاه در کیتوزان تحت بسته‌بندی اتمسفر اصلاح شده

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چکیده

غلات تازه جوانه‌زده به‌دلیل فواید سلامتی بخشی که دارند، منبع غذایی محبوب و پرطرفدار به شمار می‌روند. آنها بسیار حساس بوده و در طول حمل و نقل، فرآوری و نگهداری بسیار مستعد به فساد میکروبی هستند. هدف از این پژوهش بررسی تأثیر پوشش نانوامولسیون حاوی کیتوزان (۰/۷۵-۰ درصد) و اسانس زرین گیاه (۳۰۰-۰ ppm) در زمان غوطه‌وری (۱۰-۱۲۰ ثانیه) بر خواص میکروبی گندم تازه جوانه‌زده طی نگهداری در دمای ۴ درجه سانتی‌گراد می‌باشد. مدلسازی اثر متغیرهای مستقل بر کیفیت محصول توسط روش سطح پاسخ انجام شد. نتایج نشان داد که افزایش غلظت اسانس و محلول کیتوزان باعث کاهش فساد میکروبی محصول می‌شود. غلظت بالای اسانس زرین‌گیاه جمعیت قارچی را پس از ۱۲ روز کاهش داد. پوشش‌دهی گندم جوانه‌زده در سطح بهینه متغیرهای مستقل (۰/۶۲ درصد کیتوزان، ۵۷ ppm، و ۲۹/۴۹ ثانیه زمان غوطه‌وری) موجب کاهش معنی‌دار جمعیت میکروبی و قارچی محصول گردید. این تیمار توانست افت وزن را کم کند و سفتی بافت، محتوی فنول کل و اسید آسکوربیک گندم جوانه‌زده را در طول دوره نگهداری سرد حفظ کند، بدون آنکه تأثیری بر خواص حسی آن داشته باشد. یافته‌های این تحقیق نشان داد که پوشش نانوامولسیونی مبتنی بر کیتوزان و اسانس زرین‌گیاه در سطوح مناسب می‌تواند در حفظ کیفیت و افزایش عمر ماندگاری گندم جوانه زده مفید و مؤثر باشد.

واژه‌های کلیدی: آنالیز میکروبی، بادرنجبویه دنایی، پوشش خوراکی، روش سطح پاسخ

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