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Effect of Allium Jesdianum's extract on the physicochemical, antioxidant, antimicrobial and sensory properties of Sausage characteristics

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ABSTRACT

The study investigated the use of Allium Jesdianum plant extract as a natural preservative in sausage dough at varying concentrations. After preparation, chemical and microbial tests were conducted on the samples at zero, 14, 28, and 42 days. The study found no significant changes in pH, moisture, fat, or protein content, but the control samples consistently had the highest total volatile nitrogen (TVN) levels. The peroxide test revealed a significant difference between the control and extract samples. Sensory analysis indicated a significant difference between the control and the 200 and 300 ppm extracts ($P < 0.05$). Addition of Jesdianum extract significantly reduced the total viable count and psychrophilic bacteria compared to the control, subsequently extending the shelf-life of the product to over 42 days. Overall, Allium Jesdianum extract, with its antioxidant and antimicrobial properties, is beneficial in preserving sausage products and can be recommended as a nitrite substitute.

1. Introduction

Sausage is one of the most popular meat products, loved by millions of consumers worldwide. In response to consumers' desire for natural products and their willingness to pay more for such foods (Marvimoghadam et al., 2022), the meat industry is seeking natural solutions to reduce oxidative stress and increase the shelf life of its products (Seifi & Khani, 2017). Fat oxidation is a crucial factor as it diminishes the quality of meat products and negatively impacts sensory characteristics like color, smell, texture, taste, and nutritional value. Additionally, it can have adverse effects on the human body. Therefore, in order to ensure consumer health and for economic reasons, extensive research has been conducted to enhance the stability of fat and fat-containing products. Utilizing antioxidants is one of the simplest methods to decrease fat oxidation (Rezagholizade-Shirvan, Shokri, Dadpour, & Amiryousefi, 2023; Sharifzadeh & Mootamednejad, 2020).

Due to the industrialization of society and people's inclination towards instant and processed foods like sausages, the consumption of these products has increased compared to the past. They are now widely consumed by millions of people worldwide (Alirezalu et al., 2018). Therefore, enriching them as much as possible would be beneficial in improving the health of individuals and society. Sausage is a type of ready-made food prepared from minced meat, animal fat, salt, spices, and sometimes aromatic plants. Typically, sausages are encased in some form of casing, traditionally made from animal intestines but now commonly made from collagen, cellulose, or even plastic (Mansour and a. R. S., 2021; Seilani et al., 2021).

In recent years, the consumption of these products has notably increased due to changes in people's lifestyles and eating habits (Bernela et al., 2014). Meat products spoil due to microbial growth and chemical spoilage. The most common form of chemical spoilage is oxidative degradation. These products have low oxidative stability and are sensitive to fat rancidity during production and storage. Much research has shown that fat oxidation in meat and meat products can be controlled or minimized by using antioxidants (Babaei Asl & Atzadeh, 2018). Nowadays, the use of nitrite as an essential component is common in the production of meat products. This compound prevents the oxidative rancidity of fats, likely due to the presence of iron in myoglobin as a pro-oxidant agent. Although nitrite performs these tasks well, it is important to minimize the intake of these substances in the diet due to the

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formation of mutagenic and carcinogenic nitrous compounds (Chamandoost et al., 2016; Yazdanfar et al., 2023). Spices and herbs are excellent sources of natural antioxidants and can serve as a great alternative to conventional synthetic antioxidants (Altememy et al., 2022a; Altememy et al., 2022b; Bahmani et al., 2022; Darvishi et al., 2022; Falahi et al., 2019; Kovsari et al., 2024; Manzoor et al., 2022; Rezagholizade-Shirvan et al., 2023; Saki et al., 2023; Saki et al., 2022; Shahzamani et al., 2023).

Ben Surh plant, also known as Allium jesdianum, Allium jesdianum Boiss belongs to the family Alliaceae and is an endemic species of Iran that grows wild in the Zagros Mountains range in southwestern Iran. The indigenous people of Iran use the leaves and bulbs of A. jesdianum for treating colds and kidney problems. Allium jesdianum contains antioxidants such as phenolic compounds, flavonoids, and flavonols, which contribute to its antioxidant activity (Pirbalouti, 2019).

Many studies have shown the antioxidant, antimicrobial, antiviral, anti-inflammatory, and anti-cancer properties of essential oils from spices. The essential oils of spices contain phenolic diterpenes that act as antioxidants and exhibit a high synergistic effect with other antioxidants. They are complex mixtures of hydrocarbons, alcohols, esters, aldehydes, and carboxylic compounds, and in some cases, phenylpropanoids. The antimicrobial properties of these oils are attributed to the presence of phenolic compounds. It is known that the antimicrobial activity of essential oils in food systems is reduced compared to the laboratory environment due to the presence of fats, carbohydrates, proteins, and salt, and different pH values. Therefore, larger amounts of these compounds need to be used in food systems, which can alter the sensory characteristics of the product (Abdi-Moghadam et al., 2023; Alirezalu et al., 2019; Darvishi et al., 2022; Rezagholizade-Shirvan et al., 2023; Rezagholizade-shirvan et al., 2023a; Rezagholizade-shirvan et al., 2022; Sadighara et al. 2016; Shokri et al., 2023).

Although numerous studies have been conducted on the antifungal and antibacterial properties of plant extracts and essential oils, few studies have reported on the activity of Allium Jesdianum plant in food systems (Gholami et al., 2016).

No study has been conducted to investigate the effect of Allium Jesdianum extract on the quality and microbial properties of susage. Considering the harmful effects of nitrite and nitrate commonly used in meat products, as well as the observed antioxidant and antibacterial effects of Allium Jesdianum plant extract it was decided to investigate the antioxidant, physichochemical, sensory properties and the antimicrobial effectiveness of Allium Jesdianum plant extract in sausage production. .

2. Method

2.1. Materials

Acetic acid, chloroform, magnesium oxide, boric acid, DPPH (2,2-Diphenyl-1-picrylhydrazy and hydrochloric acid were manufactured by Merck (Frankfurter Strasse, Darmstadt, Germany), while iodopotasium and sodium thiosulfate were obtained from Applie Chem in Germany. Allium Jesdianum plant or Ben Sorkh plant was sourced from Attari in Neishabur city.

2.2. Extraction by percolation method

For extraction, the plant (onion and stem) underwent washing, drying, and crushing with a mill to obtain the extract. After sieving, the extract was mixed with ethanol solvent at a ratio of 1:10 and placed on a hot plate at 250 rpm for 24 h at ambient temperature. Subsequently, it was filtered using Whatman No. 1 filter paper. The solution was concentrated by a rotary evaporator at 40 °C, and the extracts were finally dried using a vacuum bed dryer at 45 °C. The dried extracts were stored in a closed, impermeable container at 4 °C until use (Liu Deng-Cheng et al., 2009; Roby et al., 2013).

2.3. Method of preparing Sausages containing extract

After preparing Allium Jesdianum plant extract at a sausage production facility in Neishabur city, sausage samples were created with varying concentrations: a control sample with 120 ppm sodium nitrite (without extract), 100 ppm extract +100 ppm sodium nitrite, 200 ppm extract +60 ppm sodium nitrite, and 300 ppm Allium Jesdianum plant extract (without sodium nitrite). The extract was added to the standard sausage mixture, and after preparation, the samples were transferred to the refrigerator. Tests were conducted at time zero, 14, 28, and 42 days after production. Each sample underwent three tests. It's important to note that the sausage formulation included red meat, liquid oil, ice cubes, salt, starch, spices, and sodium nitrite additive (Jongberg et al., 2013; Kurćubić et al., 2014; Liu DengCheng et al., 2009).

2.4. Chemical tests

2.4.1. Measurement: pH

Initially, a portion of the sample was homogenized using a shredder. Five grams of the homogenized sample were added to 50 cm^3 of cooled, boiled distilled water free of carbon dioxide. The mixture was thoroughly stirred for 30 s. A digital pH meter (Multiline P4 WTW) was calibrated using buffer solutions of pH 4 and 7. The pH electrode was placed in the sample, and the pH was recorded as 20.

2.4.2. Protein percentage measurement

A precise amount of 10–9 g of the homogenized sausage sample was weighed into the digestion flask. Then, 8.5 g of digestion catalyst (100 g of potassium sulfate +10 g of copper sulfate +1 g of selenium powder) and 20 cm^3 of pure sulfuric acid were added. The end of the digestion flask was sealed with a gas absorber, and the flask was heated in a specialized oven. The temperature was initially kept low, and after half an hour, it was increased until the contents of the flask became completely colorless. Once the flask cooled, 250 cm^3 of distilled water were added, followed by the addition of 50 ml of 50% to 30% sodium hydroxide. A glass pearl and a few drops of 1% phenolphthalein in ethanol were added. The flask was then connected to an open cold water tap. In a container containing 10 ml of 2% boric acid and a few drops of totyrol reagent, the flask was placed at the cold end so that the end of the tube was submerged in the solution. Thirty to 50 % soda was added drop by drop to the flask from the device's funnel until the contents of the flask became completely alkaline, creating a pink color. Considering that each cubic centimeter of 1% normal acid is equal to 0.0014 g of sample nitrogen, the percentage of nitrogen was calculated using the following formula (Hidayat et al., 2018).

$$\text{(N)percent sample nitrogen} = \frac{\text{amount of acid consumed is 1\%normal} \times 0/0014 \times 100}{\textit{Sample amount}}$$

2.4.3. Fat measurement

A specific amount of the homogenized sample was combined with sand and sous, and then mixed for 6 h at a temperature of 100 degrees Celsius (1.5 h at a temperature of 125 degrees Celsius) to thoroughly eliminate its moisture. Subsequently, the contents were transferred into a cartridge and connected to the tube of the Soxhlet distillation apparatus. The apparatus, accurately weighed (A), was filled with 250 cm^3 of chloroform. It was then connected to the distillation apparatus, including the connecting pipe and refrigerant. The cold water inlet valve was opened, and the distillation process continued until the fat was completely extracted. The chloroform solution in the flask was evaporated entirely using a rotary device or an oven. After evaporation, the flask was reweighed (B). The total amount of fat in the sample was calculated using the following equation (Sadeghi et al., 2022).

$$\text{Fat percentage} = \frac{(B - A) \times 100}{\textit{Sample amount}}$$

2.4.4. Measure the humidity percentage

The empty Chinese kosol was placed in an oven at 100 degrees Celsius for one hour and then cooled in a desiccator. The sample, which was completely homogeneous and uniform, was accurately weighed using a specialized device and placed into the Chinese kosol. It was then weighed again (B). If the two weights differed, the process was repeated until a consistent weight was reached. The moisture percentage was calculated using the following equation (Alirezalu et al., 2018).

$$\text{Moisture percentage} = \frac{(A - B) \times 100}{\textit{Sample amount}}$$

2.4.5. TVN - test

To conduct this test, was followed the method outlined by Pirouti et al. (2014). Ten grams of sausage were thoroughly mixed with 50 ml of distilled water, and then 200 ml of distilled water were transferred into automatic tubes. Additionally, 2 g of magnesium oxide and a drop of oil were added as an anti-foaming agent. A 250 ml Erlenmeyer flask, containing 25 ml of 3% boric acid and methyl red reagent, was positioned under the apparatus. The distillation process continued until a volume of 150 ml was collected. The content of the Erlenmeyer flask was titrated with 0.1 normal hydrochloric acid, and the TVB-Na concentration was calculated using the provided formula (Pirouti et al., 2014).

$$\%\text{mg TVB} - \text{N} = (V \times C \times 14 \times 100)/10$$

2.4.6. Peroxide (PV) test

In oxidation experiments for peroxide measurement, 20 cc of Decatur's lower phase, used for sausage fat extraction, was carefully transferred to a 250 ml Erlenmeyer flask. Then, about 25 cc of a chloroform-acetic acid solution (in a 3:2 ratio of chloroform to acetic acid) was added to the flask. To this mixture, 0.5 cc of potassium iodide solution saturated with 30 cc of distilled water and 0.5 cc of 1% starch solution were added. The amount of released iodine was standardized with 1% sodium thiosulfate solution.

2.4.7. DPPH assay

Antioxidant Assessment: The ability of the ethanol extract to scavenge DPPH radicals was evaluated following the methodology outlined by Ghasemi Pirbalouti (2019). Extracts (100 μl) ranging in concentration from 100 to 300 μg/ml were mixed with 3.9 ml of a 0.2 mM ethanol solution containing DPPH (Sigma-Aldrich Co., Steineheim, Germany) in equal parts. After a 30-min incubation at room temperature, the reduction in DPPH absorbance was measured at 517 nm using a Perkin-Elmer Lambda UV/Vis spectrophotometer, with ethanol as the baseline and DPPH without antioxidant as the control. Percentage inhibition was calculated using the formula: (AC0 - AAt) / AC0 × 100, where AC0 is the initial absorbance of the control at $t = 0$ min and AAt is the absorbance of the antioxidant at $t = 30$ min. Each measurement was performed in triplicate for accuracy (Ghasemi Pirbalouti, 2019).

2.5. Microbial test

2.5.1. Total number of microorganisms (total viable count)

Initially, under sterile conditions, 25 g of the sample were mixed with 225 ml of physiological saline or sterile distilled water in a Stomaker to obtain a dilution of 0.1. Additional dilutions of 0.01 and 0.001 were prepared in the same manner. One milliliter of each dilution was transferred to a sterile plate using a sterile pipette, and molten nutrient agar at 40 degrees Celsius was added. After sealing the agar plates, they were placed in an incubator at 37 degrees Celsius for 48 h. Colonies were then counted using a colony counter, and the results were expressed in terms of colony-forming units (cfu) per gram of the sample, compared with the allowable limit (Karimpour et al., 2021).

2.5.2. Total aerobic psychrotrophic count

All conditions mentioned in the method for determining the total number of microorganisms remained valid in this experiment. Instead of the pour-plate method, the surface plating method was employed. 0.1 ml of the prepared dilutions was applied to nutrient agar plates using an L-shaped glass rod. The plates were then kept at 4 degrees Celsius for one week, and readings were conducted (fakhimi, Motamedi et al. Fakhimi et al., 2021).

2.5.3. Sensory evaluation

Different treatments were used to prepare sausages, and their sensory characteristics, such as appearance, aroma, taste, and overall desirability, were evaluated by 15 semi-trained individuals using a hedonic test framework. These evaluators, which included professors and students with experience, randomly evaluated the sausage samples. Their opinions were recorded on an evaluation sheet, and they were instructed to drink water between samples to cleanse their palate (Paulos et al., 2015).

In this research, the appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of the research and who provided the permission, and also the sensory panel participants have given permission to participate and use their data/answers.

2.5.4. Sensory evaluation questionnaire of Sausages

Evaluators were asked to assess the sausage samples for specific sensory attributes using a 5-point scale (1 to 5, indicating unacceptable, relatively satisfactory, good, very good, and excellent). They were also advised to drink water before each taste evaluation.

2.6. Statistical analysis

The tests were repeated three times and all analyses were performed in triplicate. Experiments were conducted in a randomized factorial design, and the data obtained were analyzed using SPSS ver.19 software. Results are expressed as mean ± standard error. Duncan's multi-range test was utilized at 0.05 of significance ($p < 0.05$) to compare the averages of antimicrobial effects and their percentages during the retention time on the investigated traits. Duncan's test was also used to compare the average scores of judges on the 5-point hedonic scale.

3. Results and discussion

3.1. pH

The pH of the food substance is related to the chemical and microbial reactions. These reactions ultimately lead to a reduction in the quality of the food. There was no significant difference in the pH of the samples among the different treatments ($p > 0.05$). Overall, pH changes were observed in all treatments during the 48 days of storage. The addition of compounds with low pH results in a decrease in pH, but the buffering properties of meat, from the presence of various protein compounds, limit the extent of pH changes. Therefore, the decrease in pH can be attributed to the survival and slow growth of lactic acid-producing bacteria during and after product production.

All the different concentration of Allium Jesdianum plant extract on day zero of product storage showed similar pH values compared to the control sample, with the highest value observed in treatment D on day 28. The most significant decrease in this index was noted in treatment C on day 28 of storage, likely due to the production of organic acids by bacteria, as well as the oxidation of fat and the formation of acidic compounds, resulting in a decrease in the product's pH.

According to Alpas, Yeni, Soyer, and Fletcher (2017), elevated pH levels lead to enhanced microbial development, making changes appear faster. Initially, during the initiation of protease synthesis by bacteria, the utilization of amino acids as a medium for growth commenced, instead of using glucose, resulting in an increase in pH due to the formation of ammonia and amines.

Research by Sindelar, Cordray, Sebranek, Love, and Ahn (2007) has indicated that the pH value is influenced by the nitrite formed in the extract powder (Sindelar et al., 2007). This decrease in pH, caused by the production of lactic acid from bacterial metabolism, inhibits the growth of coliforms and extends the shelf life of the product.

Additionally, Sindelar et al. (2007) (Sindelar et al., 2007) demonstrated that the pH of meat decreased following the addition of plant powder (Fig. 1 A and B).

3.2. Changes in humidity within treatments

Fig. 2 A and B illustrates the changes in moisture content among the various treatments. As anticipated, a significant difference ($p < 0.05$) was noted in the moisture content of the samples treated with Allium Jesdianum plant extract compared to the control sample. High moisture content in a meat product suggests that water has partially replaced essential nutrients. Drying, as expected, led to a decrease in moisture content (weight loss) of the sausages over time, although no significant numerical difference was observed. Lower moisture content is noticeable in products with high extract content during the storage period.

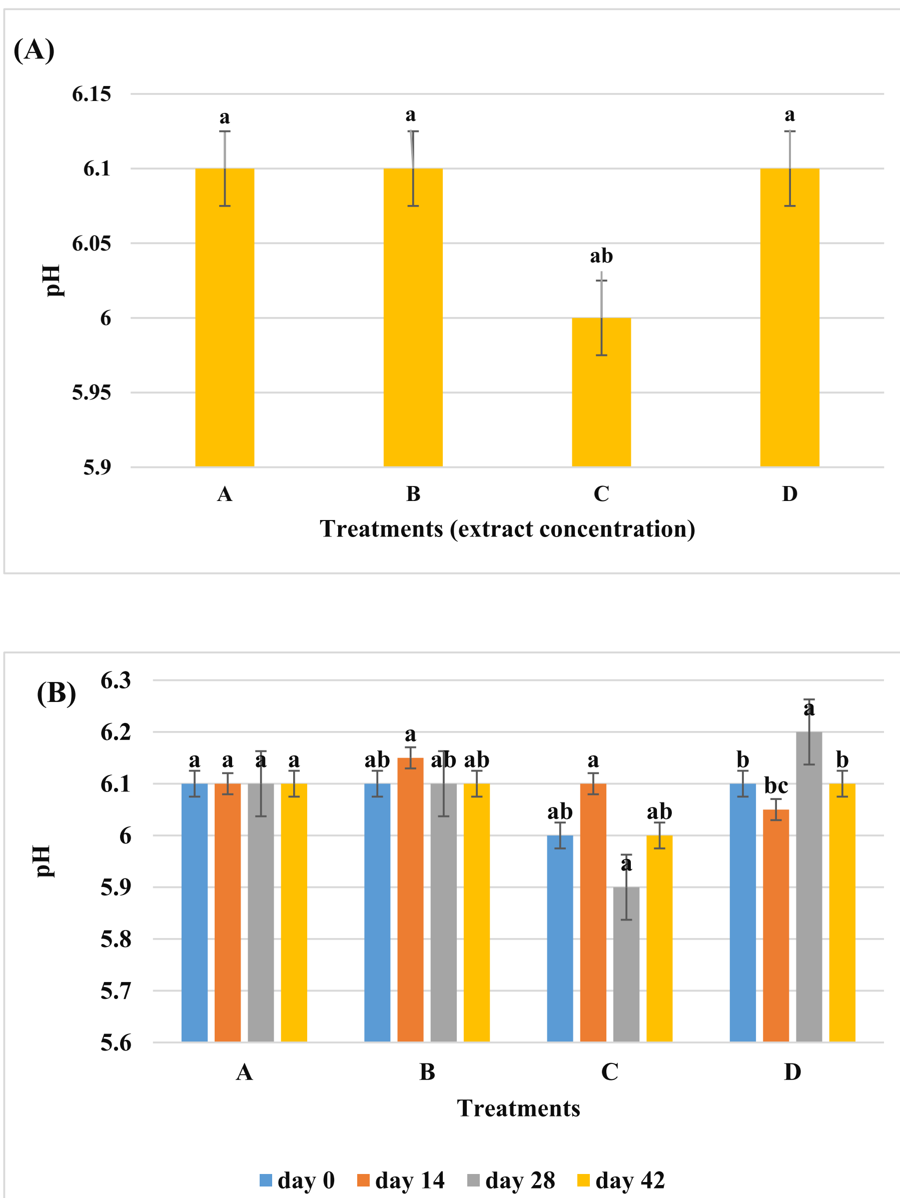

Fig. 1. The trend of pH changes in sausage samples containing extract (A), pH changes in sausage samples containing extract based on the storage period(B).

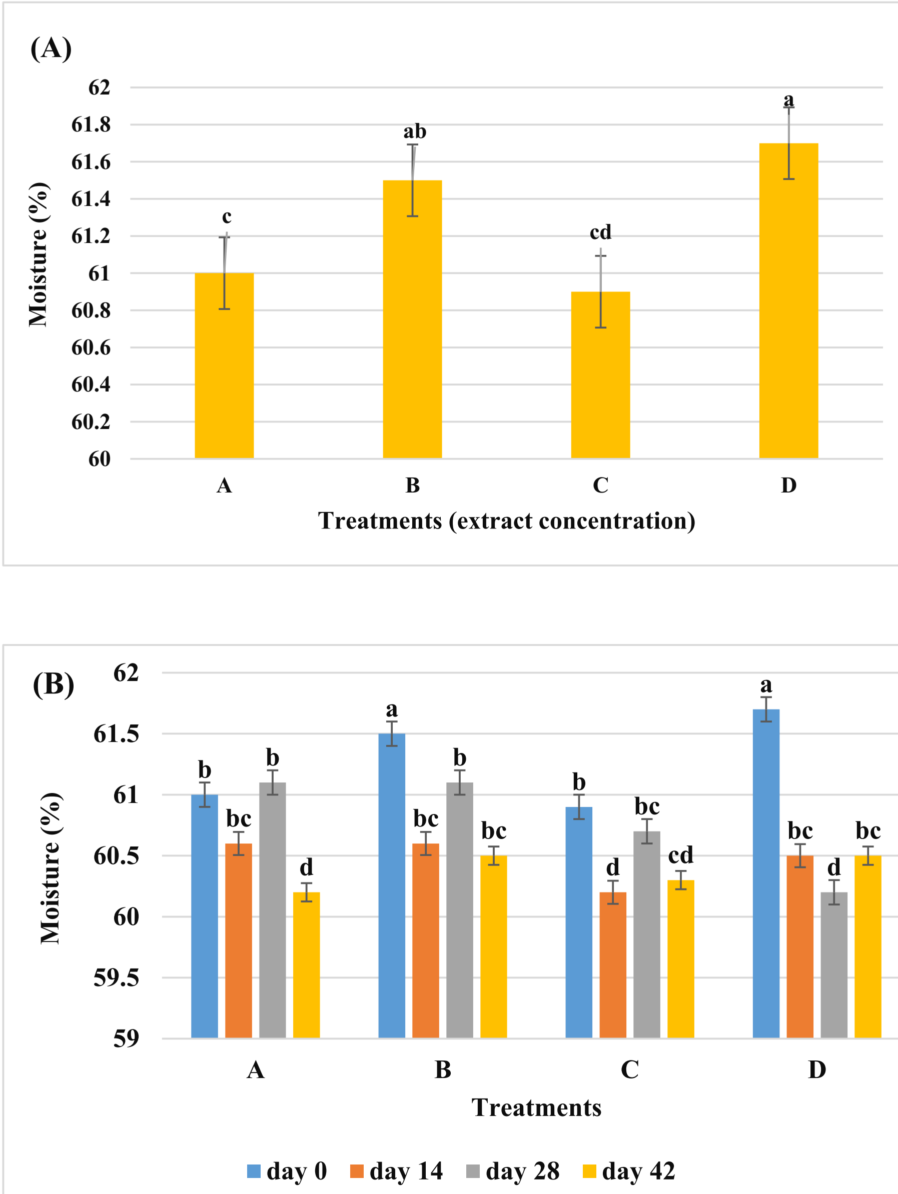

Fig. 2. The changes in moisture content of sausage samples containing extract (A), The changes in moisture content of sausage samples containing extract during the storage period(B).

Consistent with this, Khaleghi, Rahbarizadeh, Ahmadvand, Malek, and Madaah Hosseini (2016) also demonstrated a decrease in moisture content in sausages treated with tomato pomace compared to the control sample (Khaleghi et al., 2016). Similarly, Ozaki et al. (2021) found that incorporating radish and beet extract powder as a substitute for nitrite reduced moisture content in fermented sausages (Ozaki et al., 2021). Predictably, moisture reduction (weight loss) of sausages occurred over time due to evaporation during drying under storage humidity conditions.

3.3. Evaluation of fat and protein in treaties

According to Fig. 3, storage time and treatment significantly impacted the fat parameter ($P < 0.05$). The data revealed that on the first day, the highest average fat content was found in the sample treated with 100 ppm sodium nitrite +100 ppm extract (B). Evaluation of the control sample over time showed numerically different, but non-significant effects ($P > 0.05$). The lowest fat content was observed in the control sample on day 42 and in the 100 ppm extract sample (B) on day 28, which was significantly different from the other samples during the storage period ($P < 0.05$). Fat levels fluctuated over time, with the exception of treatment B, which showed a decline. The lowest and highest fat contents on day 42 were in the control sample (A) and the 100 ppm sample (B), respectively. Generally, as the levels of the plant extract increased, the fat content in the sausage decreased, although this process was not entirely consistent.

According to Fig. 4, the process of changing protein levels showed a declining trend. The lowest and highest amounts of protein were reported on day 42 in the control sample (A) and on day 1 in the 100 ppm extract sample (B) with the numeric values of 16.17% and 57.17%, respectively, which were significantly different ($p < 0.05$). On day 1, the protein content of the control sample and the 100 ppm extract sample (B) differed significantly ($P < 0.05$). Sample B showed the highest protein level, differing significantly from the other samples ($P < 0.05$) each day. Between the two treatments A and D, belonging to the control group and 300 ppm plant extract without sodium nitrite, there was no

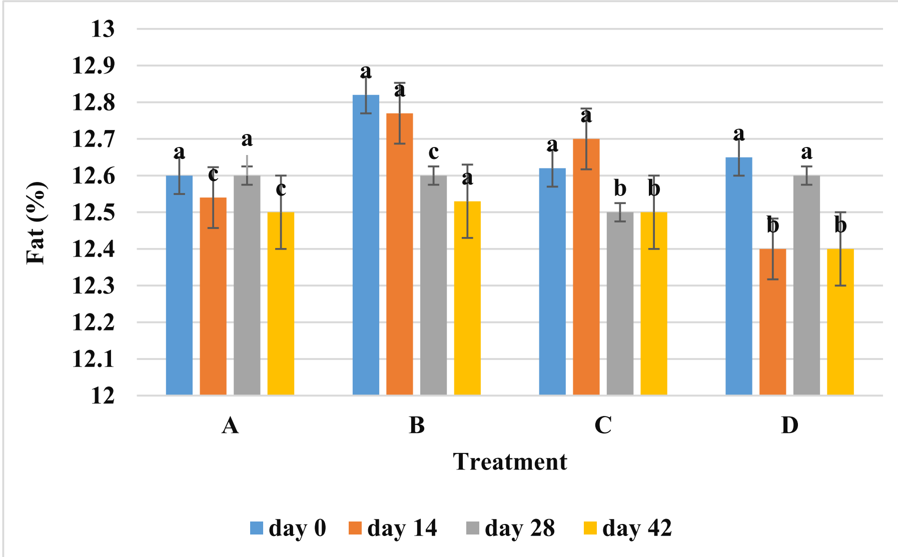

Fig. 3. Fat Change trends in samples of sausage containing extract.

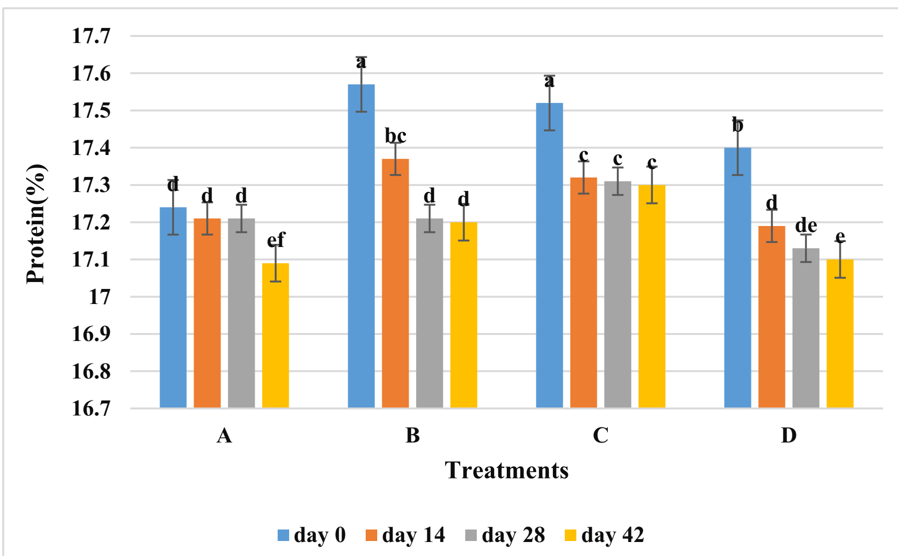

Fig. 4. The process of protein changes in sausage samples containing extract.

significant difference ($p > 0.05$) in protein levels. The protein levels decreased over time, and at the end, the lowest protein levels belonged to the control sample and 300 ppm extract without sodium nitrite. In contrast, treatment C containing 200 ppm extract +60 ppm sodium nitrite showed the highest protein level.

Fat oxidation, as a rancidity agent, is a critical factor in reducing the quality of meat and meat products. This is primarily due to the high fat content and long-term storage of these food items. This process leads to increased oxidation of proteins, color changes, and ultimately, textural and tissue defects in the meat products. The oxidation of proteins, such as myoglobin, results in the production of brown metmyoglobin, which negatively impacts the appearance of the meat and meat products (Sharifzadeh & Mootamednejad, 2020). According to Williams et al. (2017), the undesirable color caused by the formation of brown metmyoglobin led to a 4.5% reduction in red meat sales and a 9.1% reduction in processed meat products (Wijegunawardhana et al., 2021).

3.4. Evaluation of peroxide index

Oxidation results in the production of peroxide or active oxygen, which is the primary product of oxidation. Hydroperoxides are unstable and break down into various compounds that can cause unpleasant odors and staleness in the product (Karimpour et al., 2021).

The peroxide index displayed significant changes over time ($p < 0.05$). All treatments exhibited lower peroxide index values during storage compared to the control sample. The lowest value was observed in the 200 ppm treatment (D - extract +60 ppm sodium nitrite) on day 28. However, the index began to increase over time and reached its peak value on day 42.

Alina, Babji, Maznah, Syamsul, and Muhyiddin (2012) measured peroxide levels in smoked chicken sausage and noted an increase in peroxide value during storage, but the presence of antioxidants can slow down this process (Alina et al., 2012). Considering that the acceptable peroxide value for fatty foods like sausage is set at 25 meq O2/kg, and all samples remained below this limit until the end of storage, it indicates

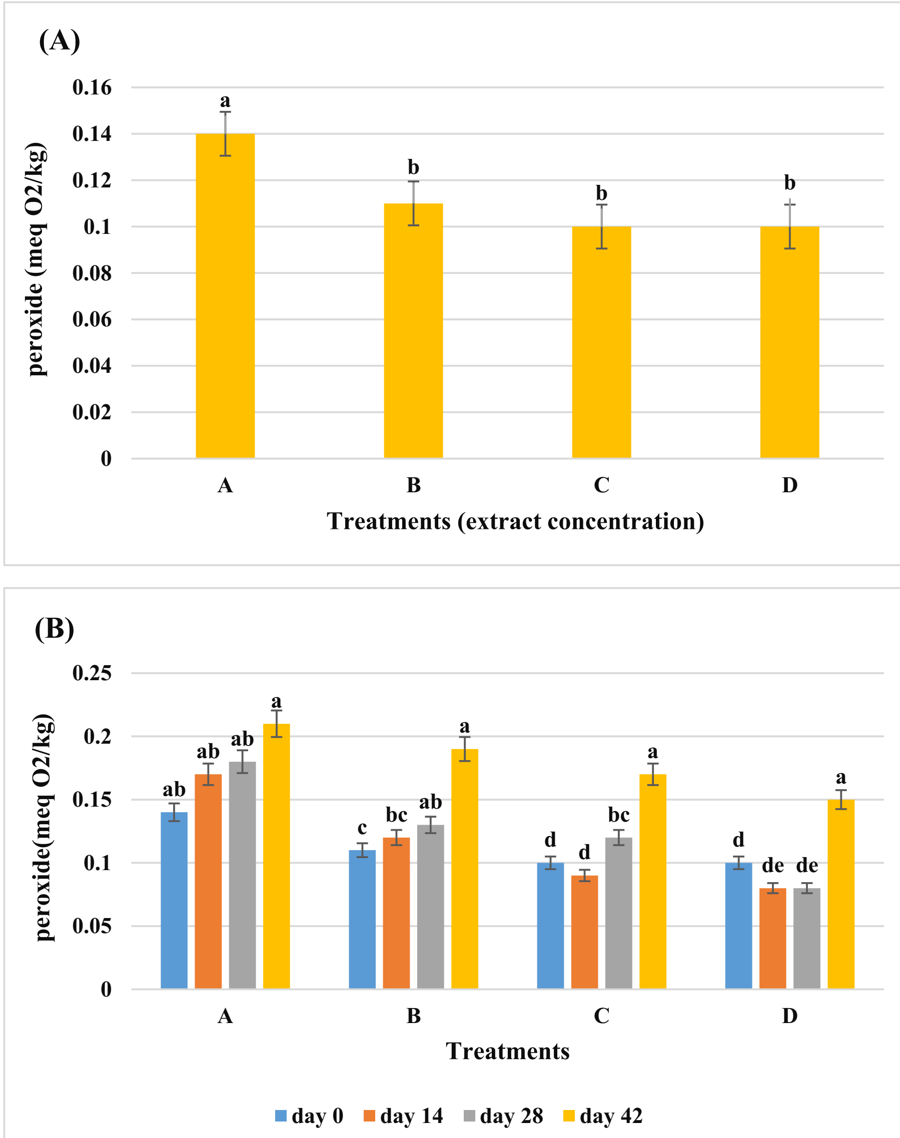

Fig. 5. The peroxide index changes in the sausage samples containing the extract(A), The peroxide index changes in the sausage samples containing the extract during storage(B).

the extension of the oxidation period and reduction of hydroperoxide breakdown (Gassara et al., 2016). This suggests the acceptability of all treatments containing Allium Jesdianum plant extract in terms of peroxide value, based on pH, fat content, and the presence of antioxidant compounds (Fig. 5 A and B).

3.5. *Examination of color changes*

The colorimetric results are shown in Table S1, and all the examined indices (L*, b*, a*) showed significant effects of different levels of Allium Jesdianum plant extract and time ($p < 0.05$ for all treatments and levels). On day zero, Allium Jesdianum plant extract showed higher a* values (redness) than the control sample. Until day 42, a significant increase in a* was observed in all treatments, but a decreasing trend was seen for the control. Liu, Tsau, Lin, Jan, and Tan (2009) also reported increased a* during storage when adding rosemary and Chinese mahogany to chicken sausage (Liu et al., 2009). Meanwhile, some researchers have reported significantly decreased redness in meat product samples during storage, due to oxidation of red pigments (Ahn et al., 2002; Liu et al., 2009).

The highest a* was seen in treatment B on day 28, and the lowest in treatment D (300 ppm extract) on day 42. Differences in yellowness relate to the presence of pigments and are not indicative of oxidation (Al Marazzeq et al., 2015). Al Marazzeq et al. (2015) reported the lowest yellowness during storage for the control with 120 ppm nitrite, and sausage samples with 80 ppm and 60 ppm nitrite plus olive leaf extract. As seen, storage time did not greatly affect changes in yellowness (Al Marazzeq et al., 2015; Das et al., 2010). Horsch (2013) noted that storage time has no significant effect on increasing or decreasing yellowness (Horsch, 2013).

The luminosity factor L* in meat products depends on pigment concentration and type, moisture content, and moisture absorption properties of dissolved substances (82). Results for L* showed that with increasing Allium Jesdianum plant extract, brightness increased. All extract levels showed higher L* on day 1 compared to the control, with the highest in treatment D (300 ppm) on day 42. Over time until day 42, L* increased in all treatments, unlike fluctuations at the end.

Liu et al. (2009) reported no significant difference in L* during 14-day storage when adding rosemary and mahogany to chicken sausage (Liu et al., 2009). In Khaleghi et al. (2016), all treatments with black barberry replacing nitrite had lower L* than the control, contrary to the present findings (Seifi & Khani, 2017). However, Al Marazzeq et al. (2015) saw increased L* over time, with nitrite plus olive leaf extract being more transparent than the control, aligning with the present results (Al Marazzeq et al., 2015; Riyad et al., 2018).

3.6. TVN

Total volatile basic nitrogen (TVB-N) serves as a crucial quality parameter for avaluation of the freshness of meat products. TVB-N is associated with protein and amine degradation during storage, reflecting spoilage progression in meat products (De Mey et al., 2017). The TVN test revealed no consistent trends among samples with varying extract concentrations. Concentration had a significant impact on TVN ($p < 0.05$), with the highest levels observed in the control sample on day 1, significantly different from the three treatments. However, the three concentrations did not show significant differences from each other ($p >$ 0.05) until day 28. With the exception of the control, all other samples exhibited a downward trend until day 28, suggesting that Allium Jesdianum showed a significant effect on this index, ultimately improving quality. By day 42, all treatments showed a significant increase in TVN (Fig. 6 A and B).

The utilization of plant extracts in meat products has a significant impact on the total volatile nitrogen index. Plant extracts, rich in bioactive compounds like polyphenols and essential oils, act as natural antioxidants, effectively reducing the formation of volatile nitrogen compounds in meat products. These extracts contribute to the preservation of oxidative stability, the stabilization of color properties, and the reduction in the production of lipid-derived volatile compounds, thereby augmenting the overall quality of the meat (Efenberger-Szmechtyk et al., 2021).

Sun et al. (2018) investigated the effect of using different spices such as cinnamon, clove, and anise on quality properties of dry sausages. Results showed a significant reduction in pH value, water activity, and

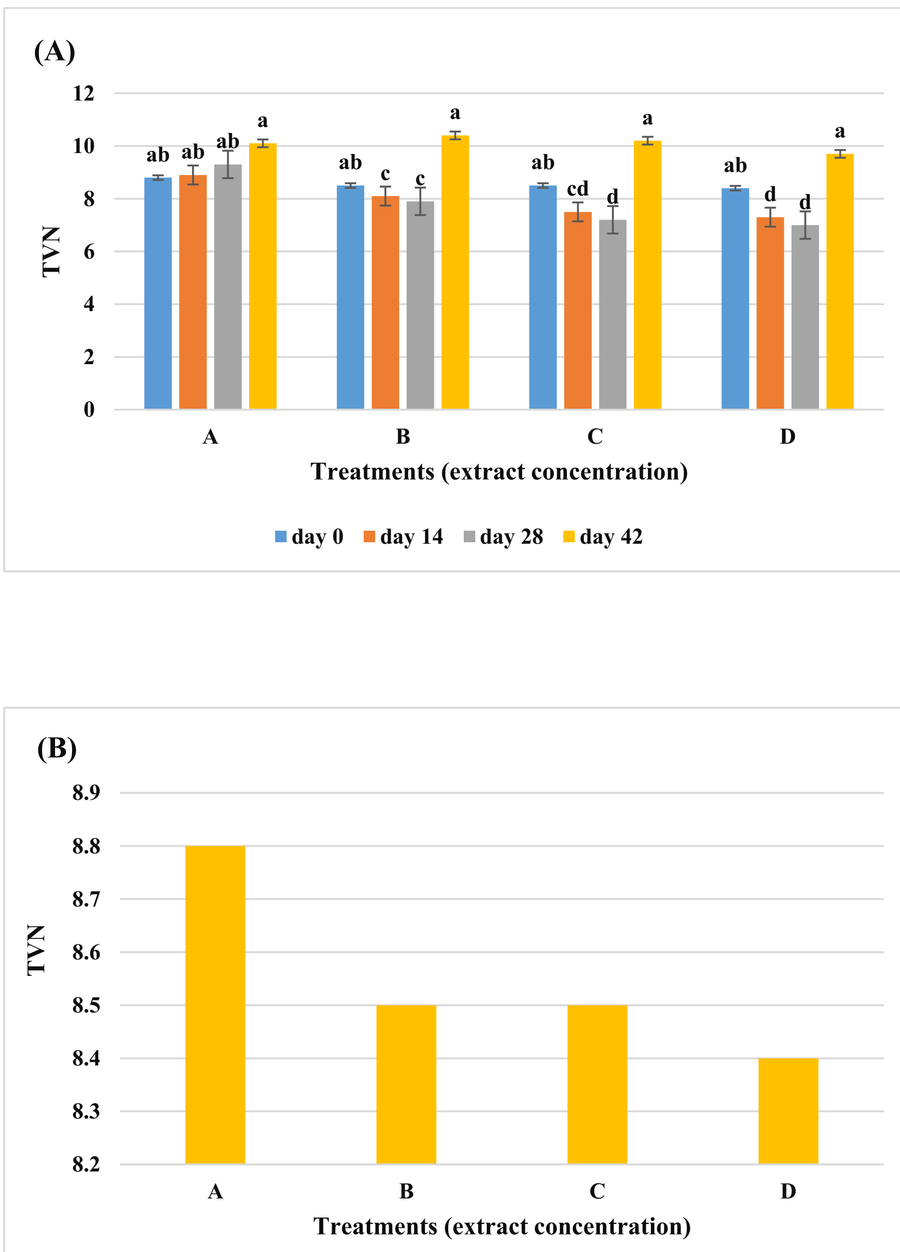

Fig. 6. Changes in TVN of sausages with different concentrations of the extract during storage days (A), the effect of the extract concentration on TVN (B).

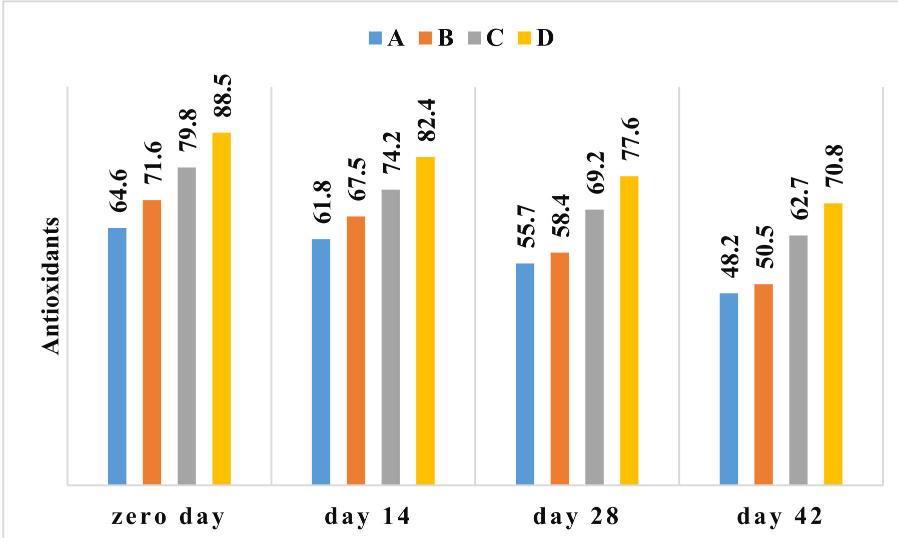

Fig. 7. The antioxidant changes in the Sausage Samples containing the extract during storage days.

total volatile basic nitrogen and the lipid oxidation was inhibited by spice extracts (especially anise) in dry sausage (Sun et al., 2018).

3.7. DPPH assay

Through a comprehensive analysis of the impact of Allium Jesdianume plant xtract on the potency of free radical inhibition, it was evident that there was a significant enhancement in free radical inhibitory activity as the concentration of the extract increased. Additionally, there was a significant correlation between time and antioxidant activity at the examined levels ($p < 0.05$). Based on the results, the highest DPPH value was demonstrated with the sausages incorporated with 300 ppm of Allium Jesdianum plant extract (70.8%). The high DPPH value in the 300 ppm extract without sodium nitrite sausage sample indicates the higher ability of antioxidant compounds in these samples to lose hydrogen and possibly act as a primary antioxidant (Fig. 7).

Yazdanian et al. (2022) revealed that the total phenolic content in ethanol extracts from the leaves and bulbs of A. jesdianum ranged between 27.83 and 98.23 mg GAE/g extract (Yazdanian et al., 2022). Ethanol extracts from the populations of A. jesdianum leaves were found to be the most effective free radical scavenging agents (Kamranfar et al., 2023). The extracts also showed moderate-to-good inhibitory activities against bacteria, especially against *B. cereus* (Alaee et al., 2021). Additionally, the elicitation of A. jesdianum calluses with methyl jasmonate (MeJ) increased the content of total phenolics, total flavonoids, total flavonols, and anthocyanin, as well as the antioxidant activity.

Ekrami et al. (2022) investigated the quality properties of bio-nanocomposite films containing Allium jesdianum Boiss for food packaging. Incorporating Allium jesdianum extracts into food products, such as meat pâté, has been found to increase the content of antioxidants and decrease the generation of secondary products of lipid peroxidation during storage (Ekrami et al., 2022).

According to Alidadi et al., 2022 study, Allium jesdianum (AJ) has been found to have antioxidant activity. The extract of AJ showed increased antioxidant activity in calluses, as evidenced by higher levels of total phenolics, total flavonoids, total flavonols, and anthocyanin (Alidadi et al., 2022). Additionally, the antioxidant fraction of *Allium cepa*, a related species, exhibited antioxidant activity with quercetin and

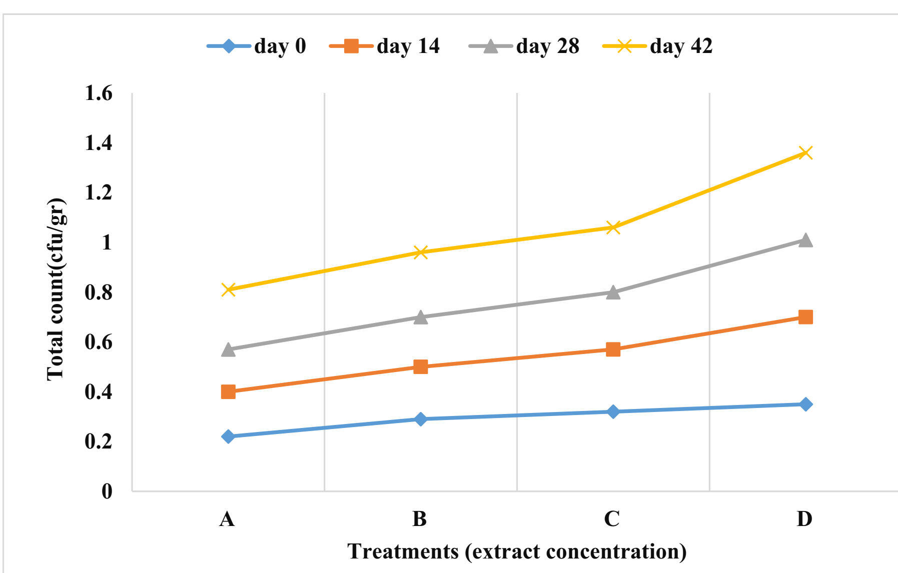

Fig. 8. Total count trend of sausage with different concentrations of extract during maintenance days.

isoquercitrin as the main constituents (Yazdanian et al., 2022). Overall, AJ shows promising antioxidant activity, and further studies can explore its potential in biotechnological applications (Shahid, Shahid, Ihsan, Anjum, & Shahid, 2022).

3.8. The effect of extract on microbial variables

An analysis of variance of the resulting data reveals a significant difference at the 0.05% level between the applied treatments and the treatment-day interactions. This information is displayed in Table S2.

3.9. Total microbial count

The changes in total microorganisms showed that the control sample gradually increased over time, but a significant increase was observed from day 28 to 42. Overall, the trend was fairly consistent across days. The control treatment had the lowest count on day 28, and on day 42 all treatments except 300 ppm plant extract without sodium nitrite had the highest amount, as shown in Fig. 8. The average comparison using Duncan's test also indicated that the control sample increased over the investigated days. Overall, the trend for total microbial count and psychrophilic bacteria showed an initial increase followed by a decrease, eventually reaching near zero. This can be considered a form of metabolic exhaustion, an important practical phenomenon and a principle of technology that can lead to self-sterilization. This was first observed in liver sausages kept at room temperature, where survivors from the heat process were eliminated during storage. Similar behaviors were later observed with Staphylococcus, Salmonella, and yeast.

In 2014, Pirouti et al. reviewed the antimicrobial properties of Thymus vulgaria extract on sausages during storage. They found that the total microbial count increased over time with 1% thymus. However, at 2% thymus, an inhibitory effect was observed, and further increases had no significant effect (Pirouti, Javadi and Nahidi, 2014a).

3.10. Evaluation of psychrophilic bacteria

The results of the study on psychrophilic bacteria are depicted in Fig. S1. The initial population in the control group on day 1 was 1.4 log CFU/g, increasing to 9.4 log CFU/g by day 42. In all samples treated with Allium Jesdianum extract, psychrophilic counts were significantly lower ($p < 0.05$) than the control. The lowest count on day 28 was observed in the 200 ppm extract +60 ppm nitrite group at 3.3 log CFU/g. The highest count was on day 14 in the control sample at 6.6 log CFU/g.

In meat products, most spoilage by psychrophiles occurs at low temperatures. The initial count was approximately 5.5 log CFU/g, and the final population was significantly reduced compared to the control ($p < 0.05$). Psychrophilic bacteria have been identified as the cause of spoilage and quality deterioration in sausages, as most mesophiles are destroyed by cooking, while heat-resistant lactobacilli remain. Lactobacilli are mostly psychrophilic and heat-resistant, highlighting their important role in the spoilage of heated meat products.

Samira et al. (2020) found that thyme essential oil extended the shelf life of fresh sausages by reducing mesophiles, which is consistent with the findings of this study. Similar results were reported by da Silveira Firmiano et al. (2014), who showed lower aerobic mesophiles in fresh Tuscan sausages with 0.1% boldo leaf extract compared to the control. Mesophiles were significantly reduced with the extract compared to the control in the fresh sausages in this study.

3.11. Sensory evaluation

Sensory evaluation was conducted on sausages produced with varying concentrations of Allium Jesdianum extract. The analysis of variance Table S3 indicates a significant difference between the 28 and 42-day treatments, as well as samples containing 200 and 300 ppm of the extract compared to other samples, with a $P < 0.05$. The comparison of sensory evaluation means (flavor, texture, and oral desirability) using Least Significant Difference (LSD) test revealed no statistically significant difference ($P < 0.05$) between the control sample and the 100 ppm extract at a 5% level. However, there was a significant difference between the control and the 200 and 300 ppm extracts ($P < 0.05$). Additionally, there was a significant difference between the 100 and 200 ppm extracts, as well as between the 100 and 300 ppm Allium Jesdianum extracts ($P < 0.05$). Further analysis of sensory evaluation data, when questions were separated, showed a significant difference in the evaluation of oral desirability between different treatments and at different times. However, when comparing other sensory parameters, there was no significant difference in the results of the evaluators for different treatments and at different times ($P > 0.05$). This suggests that the percentages used did not impact appearance and aroma, but had a notable effect on oral desirability, which could greatly influence the marketability of the product as texture and taste are crucial factors for customer satisfaction. Plant extracts play a vital role in influencing the sensory characteristics of meat products. They possess the capacity to boost the taste, aroma, and overall palatability of meat based foods. By incorporating plant extracts rich in antioxidants, such as polyphenols and flavonoids, meat products can enhance their sensory attributes while prolonging their shelf life. These extracts are capable to prevent undesirable changes in taste and odor, ensuring a more appealing product for consumers. Furthermore, plant extracts can function as natural preservatives, substituting synthetic additives that could potentially have deleterious impacts on health (Kurćubić et al., 2023; Moustafa et al., 2021; Sun et al., 2018). Moustafa et al., 2021 indicates that the incorporation of plant extracts into susage can significantly enhance their sensory qualities, making them more acceptable to consumers (Moustafa et al., 2021). Sallam et al. (2004) observed that garlic had no significant impact on the tenderness or the acceptability scores of cooked chicken sausage during storage time (21 days) (Sallam et al., 2004). According to Boeira et al., 2020 study, no statistically significant variance ($p < 0.05$) observed between the sensory evaluation and fresh susage samples incorporating *Cymbopogon Citratus* extract in relation to the color, taste, texture, and overall acceptability of the sensor. But, in the odor attribute 1.0% extract received the highest evaluation (Boeira et al., 2020).

3.12. Texture

Except for the control sample, the evaluators evaluated other treatments containing different concentrations of extract with the same score. Statistical analysis also showed that the texture of sausages containing 100 ppm of Allium Jesdianum extract was not significantly different from sausages containing 200 and 300 ppm ($P > 0.05$).

3.13. Taste

In regards to this variable, similar to the evaluator's assessment of texture, all treatments containing different concentrations of the extract, except for the control sample, received the same score. Statistical analysis also indicated that the taste of sausages containing 100 ppm of Allium Jesdianum extract was not significantly different from sausages containing 200 and 300 ppm ($P > 0.05$).

3.14. Color

The color attribute of meat products has a significant impact on consumer perceptions and is the most important component of product quality. Consequently, a product may be rejected solely on the basis of its color before any other attributes are considered (Purlis, 2010). Color analysis was also conducted as it is a crucial factor for customers, revealing that other treatments containing varying concentrations of the extract had similar results. Statistical analysis also showed that the color of the 100 ppm extract did not significantly differ from the 200 and 300 ppm extracts ($P > 0.05$). Similarly, Sebranek, Sewalt, Robbins, and Houser (2005) observed effectiveness of rosemary extract in preserving the color in frozen pork sausage, with 2500 ppm concentration (Sebranek et al., 2005).

3.15. Oral desirability

The final parameter of the sensory evaluation of the product was completed. The extract from the onion family, similar to leek, had a distinct flavor profile with the lowest score. Samples containing 200 and 300 ppm extract at 28 and 42 days of storage received the highest score, showing a significant difference from the other samples. Overall, the addition of extract at 200 ppm improved the sensory quality of the sample, with further enhancement at 300 ppm. In a study by Maravi et al. in Fakhimi et al., 2021, sausages with peppermint extract showed no significant difference in taste compared to the control group, which is consistent with the results of this study (Moghadam, Mohammadreza, & Sharifzadeh, 1401). Similarly, Sharifzadeh. in 2020 found no significant differences in taste and aroma when using lettuce powder and extract (Sharifzadeh Ali, 2020). These findings are in line with the results of the present study.

4. Conclusion

This study concluded that Allium Jesdianum extract provide antioxidant and antimicrobial benefits to sausage during storage (42 days) and the effects are concentration dependent. All extract levels showed similar pH to the control on day 0, with the highest pH in the 300 ppm extract on day 28. PH changes occurred over 48 days in all treatments. The extract beneficially reduced moisture, with greater reductions at higher concentrations of extract. Fat fluctuated over time but mostly declined, except in treatment B. The lowest and highest fat levels on day 42 were in the control and the 100 ppm extract, respectively. Protein declined over time, with the lowest levels in the control and the highest in the 100 ppm extract on days 42 and 1. The extract showed antimicrobial effects, with 1% extract inhibiting growth. Metabolic exhaustion was implicated in the declining microbe counts during storage. The 200 ppm extract treatment had the lowest total and psychrophilic bacteria counts. The extract significantly improved the TVN index, with similar concentrations until day 28 but decreases compared to the control, indicating improved quality. All treatments had higher a* values than the control on day 0, and a* increased by day 42 in all treatments except for the control. The highest a* value was in the 100 ppm extract on day 28. L* increased with increasing extract concentration, and all concentrations were higher than the control on day 1. Therefore, it is suggested that Allium Jesdianum, as a natural herb, could be utilized to prolong the shelf-life of meat products, providing the consumer with food containing natural additives, which might be seen more beneficial compared to synthetic origin.

Ethics approval and consent to participate

According to the regulations of our institute, this activity does not require obtaining an ethic code from the institution, and people knowingly participated in this sensory evaluation panel.

CRediT authorship contribution statement

Akram Ghorbani: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Kamiar Mahmoudifar:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Samira Shokri:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Yeganeh Mazaheri:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Ehsan Shamloo:** Writing – review & editing, Writing – original draft, Investigation. **Alieh Rezagholizade-shirvan:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Amir Hossein Elhamirad:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation.

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

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101461.

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