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The combined effect of *Bacillus coagulans*, potassium chloride, and yeast extract on the physicochemical and sensory characteristics of functional vegetable sausage

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ABSTRACT

This study investigated the impact of *Bacillus coagulans*, potassium chloride, and yeast extract on probiotic vegetable sausages' microbial, physicochemical, and sensory properties with reduced salt content during refrigerated storage. The sausages supplemented with potassium chloride solution and 1% or 2% yeast extract showed an initial viable count of *B. coagulans* at 8.34 log CFU/g, which decreased to 7.2 and 7.42 log CFU/g after four weeks of storage. The addition of potassium chloride and yeast extract improved the viability of *B. coagulans* by approximately 0.2 log CFU/g compared to sausages without the mixture. Moreover, including these ingredients led to a gradual decrease in pH over the storage period. Sensory evaluation indicated that adding potassium chloride and yeast extract enhanced taste scores and overall acceptance but negatively impacted color characteristics. These findings demonstrate that substituting salt with potassium chloride and incorporating yeast extract enhances the survival of probiotic *B. coagulans*, lowers pH during refrigerated storage, and improves sensory attributes in vegetable sausages. The study suggests combining potassium chloride, yeast extract, and probiotic bacteria in plant-based meat products like sausages offers a scientific and practical approach to promoting healthier consumption patterns. However, it emphasizes the importance of carefully determining the appropriate concentration of these ingredients based on the sensory evaluation results.

1. Introduction

In recent decades, the incidence of non-communicable diseases related to food and nutrition, such as obesity, hypertension, and diabetes, has increased. This has led to a significant change in consumer demand for food products (1). The term "functional food" was first used in Japan in the 1980s to describe food products enriched with special compounds that provide appropriate physicochemical properties in the final product (2, 3). Functional foods are food and drink products that provide the energy and nutrients the body needs while also regulating one or more specific functions through a particular physiological response or reducing the risk of disease (4). Probiotic food products have captured a significant share of the functional food market. Extensive scientific evidence supports the health benefits of probiotic food products (5, 6). *Bacillus coagulans* is a gram-positive, spore-producing probiotic bacterium with an optimal growth temperature of 35-50°C and an optimal pH of 5.5-5.6. It is approved by the US Food and Drug Administration as a GRAS (Generally Recognized as Safe) microorganism, meaning it is safe to add to food without requiring pre-market safety assessment. Due to its resistance and stability against heat, pressure, and acidity, *B. coagulans* is a candidate for use in non-dairy products such as flour and meat products that undergo thermal processing. Although there are limited studies on the use of *B. coagulans* in functional foods, a wide range of commercial probiotic products currently contain this microorganism (7-9). Meat is a popular food item among non-vegetarian consumers worldwide due to its texture, taste, and

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nutritional value. However, meat has faced criticism for various reasons, including religious beliefs, health concerns, environmental protection issues, and greenhouse gas emissions. As a result, consumers are increasingly demanding reduced meat consumption. One successful strategy for reducing meat consumption is producing suitable alternative products known as meat analogs. These compounds simulate the taste, smell, texture, and appearance of meat without using meat. Although some meat analogs are available on the market, their share is very small (about 1-2%) compared to the significant market for meat products (10, 11). Despite the popularity of processed meat products among young people, they have been criticized for their potential negative health effects due to their high salt content and use of chemical preservatives. Reducing losses by replacing harmful substances with beneficial ones and turning health-related challenges into opportunities from a nutritional perspective is a novel and successful strategy for developing functional products. Excessive salt consumption has increased blood pressure and the risk of cardiovascular diseases and other health issues. Therefore, reducing per capita sodium consumption is a priority for health systems seeking to improve nutritional patterns (12, 13). The US Dietary Guidelines (2005) commented on the effect of some salt substitutes on specific groups. Generally, they stated that a potassium-rich diet can reduce the effects of salt on blood pressure. It is recommended to consume 4.7 grams of potassium per day. Despite these warnings, most studies have focused on reducing sodium in processed food products by replacing part of it with potassium chloride (14, 15). However, sensory defects related to an increase in bitter and metallic taste and a decrease in salty taste are the main limitations of using potassium chloride as a substitute for sodium chloride in meat products (16). Using compounds that can improve the sensory properties of potassium chloride, such as yeast extract, can effectively improve nutritional formulations and reduce sodium content (17). Yeast extract is a natural flavor enhancer often used as a substitute for artificial flavor enhancers such as monosodium glutamate. It contains free glutamic acid and has low sodium content. However, the amount of yeast extract used to create a meaty taste in products is limited, and excessive use can cause an undesirable side taste (14, 18). This study aims to evaluate the combined effect of *B. coagulans*, potassium chloride, and yeast extract on the physicochemical and sensory characteristics of functional vegetable sausage (probiotic and salt-reduced) over 28 days.

2. Materials and Methods

2.1. Materials

The oil used in the product formulation was ordinary soybean oil obtained from Mahidasht Kermanshah Agriculture and Industrial Complex. Pasteurized egg white powder was obtained from the Narin factory in Hamedan. Kappa-carrageenan gum, wheat gluten, and citric acid (powder form) were obtained from Beh Azma Shiraz Raw Materials and Additives Manufacturing Factory. Paprika powder, a permitted natural edible color, was obtained from Fars Mahzon Import and Trading Company of Shiraz. Refined edible salt used in the food industry was obtained from the Sapid Daneh factory. Soybean isolate was obtained from Shandong Sinoglory Health Food Co., Ltd. Yeast extract powder was purchased from Soren Tech Toos Company. The autolyzed yeast extract was derived from Saccharomyces cerevisiae yeast through enzymatic reactions. Potassium chloride was obtained from Tehran Chemical Company.

2.2. Vegetable Sausage Formulation

The formulation of probiotic vegetable sausage (control) and its different treatments are given in Tables 1 and 2.

Table 1. Formulation of probiotic vegetable sausage (control sample)

Raw material/additive	Formulation amount (%)
Edible liquid oil	14
Egg white powder	5
Kappa-carrageenan gum	3.5
Wheat gluten	5
Spices	2
Paprika powder	0.2
Citric acid	2
Refined salt	1.8
Soy isolate	0.5
Water	66

Table 2. Changes in the formulation of study treatments.

Treatment number	Yeast extract (%)	Potassium chloride (%)	Salt (%)	Presence of probiotic species
1	0	0	1.8	No
2	0	0	1.8	Yes
3	1	0.45	1.35	Yes
4	2	0.45	1.35	Yes

In samples 3 and 4, for the amount of yeast extract added, the amount of water used in the formulation was reduced.

2.3. Preparation of Studied Probiotics

This study used lyophilized powder of *B. coagulans* ATCC 7050 produced by Roshd Mehrgan Campus Biological Products Company at a 10^{13} CFU/gr concentration. To prepare the amount of BC spore inoculation, probiotic bacteria powder was first dissolved in water according to the instructions and standards stated by the factory and then added to the vegetable sausage dough. Counting was performed to ensure the initial concentration by the national standard of Iran No. 12105, "Microbiology of animal and human feed - enumeration of possible species of probiotic sporulated bacilli". The general principles of counting include preparing an initial suspension to obtain a uniform distribution of test bacterial cells, using thermal shock at a temperature of 80±2 °C for 10 minutes, incubating for 60-75 minutes at a temperature of 37 °C, preparing subsequent decimal dilutions of the suspension, cultivating successive dilutions by pour plate method, incubating plates upside down for 24-48 h at a temperature of 37 °C, and expressing results as the number of colony-forming units in one gram of the product.

2.4. Preparation of Vegetable Sausage

To prepare the control sample without probiotic bacteria and yeast extract mixture, the following method was used: The raw ingredients for 10 kg of vegetable sausage were carefully weighed according to the presented formulation. Soy protein, wheat gluten, egg white powder, salt, and other powdered additives were added to the cutter and mixed slowly for 2 minutes. About half of the water used in the formulation was added to the mixture in the form of ice powder, and the cutter began mixing the ingredients at high speed. After lowering the temperature to 1 °C, oil was added to the formulation, and the cutter operation continued until the temperature reached 8 °C. The rest of the water was added to the dough and mixed entirely at high speed (about 3 minutes) until a uniform, doughy texture was achieved. A digital laser thermometer was used to control the temperature of the emulsion and paste (keeping the temperature below 12 °C) at all stages. To prepare the inoculated formulation of *B. coagulans* ATCC 7050 spores, about 0.64 gr of probiotic spores were added to the mixture after adding half of the water in the form of powdered ice during the first step of adding ingredients to the cutter. The ingredients were mixed together at low speed for 3 minutes to distribute the spores in the paste evenly. The concentration of inoculated probiotics was 10^{13} CFU/gr. Next, a potassium chloride mixture was used instead of a quarter of the salt used in the control formulation when adding ingredients to the cutter. Yeast extract was added at amounts of 0.5% and 1% to groups 3 and 4, respectively. All samples were then filled into polyamide coatings and baked in a steam baking room at 80°C for 60 minutes until reaching a center temperature of 75°C. The product temperature was lowered to about 25 °C with a cold-water shower (12 °C) for 10 minutes and stored at 4 °C until experimentation.

2.5. Viability of probiotic bacteria in vegetable sausage

Probiotic bacteria were counted according to Iranian National Standard No. 12105 “Microbiology of animal and human feed - Counting possible species of probiotic sporulated bacilli.” To prepare the initial dilution, 10 grams of probiotic sample were added to 90±0.1 ml Ringer’s buffer solution, then heated at 80 ºC for 10 minutes in a water bath. The sample was immediately cooled in cold water to 45 ºC, then incubated for 60 minutes at a temperature of 37 ºC in a shaker incubator at a speed of 100-150 rpm before preparing subsequent decimal dilutions. One ml of each dilution was poured into two sterile plates, then dextrose tryptone agar with bromocresol at 45 ºC was poured into each plate and carefully mixed with culture medium by rotating the plate. Plates were placed on a horizontal cold surface until the mixture froze, then incubated upside down at 37 °C for 48 h. After incubation, plates with less than 300 colonies with yellow halo were selected and counted.

2.6. Chemical analysis

Analysis pH was measured according to Iran’s national standard number 1028 using a pH meter (WTW pH 530, Germany). After washing the electrode with neutral buffer, it was placed in a portion of the homogenized sample. To prepare the homogenized sample according to the relevant standard, the sample was homogenized twice by a meat grinder, and 20 grams of the sample were mixed with 2 grams of 0.1 M potassium chloride solution and homogenized by a stirrer. The fixed number on the monitor was recorded as the pH of the sample.

2.7. Examination of sensory properties

Sensory characteristics of sausage samples were evaluated on the 28th day of the study by 32 food control inspectors from the Food and Drug Organization and personnel of Shiraz University of Medical Sciences using a scoring test at the site of the Food and Drug Organization of Shiraz University of Medical Sciences. These evaluators, randomly selected based on their interest and ability to understand the evaluation method (age range 34 to 51 years and gender 18 men and 14 women), had a history of consuming sausages before starting the evaluation and were informed about how to complete the evaluation sheet. Samples from 4 formulas were randomly placed in 3 mm thick slices in colorless, odorless disposable dishes labeled with 3-digit numbers selected from random table numbers. Evaluators evaluated samples separately under daylight conditions (moonlit and sunny fluorescent lamps) at ambient temperature. Before starting the evaluation, evaluators were asked to rinse their mouths with mineral water at 20 ºC, which they did after tasting each sausage sample. The indicators determined according to table number were provided to each evaluator for each defined sensory indicator so that after evaluating samples, they could be ranked according to the indicated index. Appropriate scores for each feature were calculated such that rank 1 was considered most acceptable and rank 4 least acceptable.

2.8. Statistical analysis

Results obtained from counting probiotic bacteria and chemical analyses were analyzed using SPSS 20 software and the One-Way ANOVA method. Mean and standard deviation are the results of double repetition. Tukey’s supplementary test was used to compare means, with a difference level of 5% considered significant. Sensory characteristics were analyzed using Kruskal-Wallis statistical test. The final results were expressed as mean and standard deviation (Mean ± SD).

3. Results

In this study, the combined effect of probiotic bacteria *B. coagulans* and a mixture of potassium chloride and yeast extract (in concentrations of 1 and 2%) on the physicochemical and sensory properties of beneficial vegetable sausage (probiotic and salt-reduced) was evaluated during four weeks of storage in refrigerated conditions on days 1, 7, 14, 21, and 28. The results of counting *B. coagulans* bacteria in vegetable sausage at refrigerated conditions are shown in Fig. 1. For all groups, on all days, the difference between the average count made with the previous counting day was significant ($p<0.05$)

and the count of probiotic bacteria decreased over time for all groups. The probiotic group without the mixture of potassium chloride and yeast extract had an average counting result on day 1 starting from 8.34 Log CFU/g, which decreased over time to reach 7 Log CFU/g on day 28.

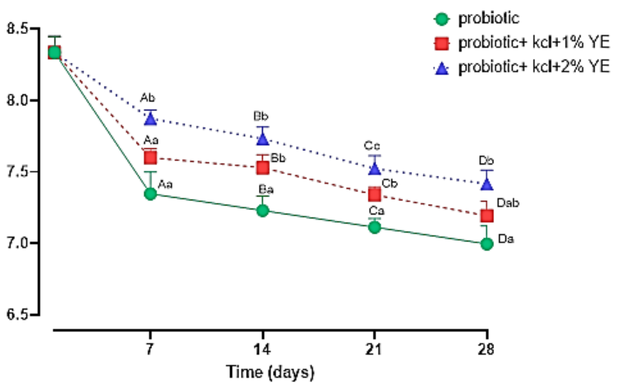

Fig. 1. The effect of the potassium chloride and yeast extract mixture on the survival of *Bacillus coagulans* in probiotic sausage during 28 days of storage at 4°C. The mean (± standard deviation) has common capital letters in each row, indicating no significant difference (p>0.05). The mean (± SD) with common lowercase letters in each column indicates no significant difference (p>0.05).

The logarithm of *B. coagulans* bacteria count in probiotic vegetable sausage containing potassium chloride mixture and concentrations of 1% and 2% yeast extract on day 28 was equal to 7.2 and 7.42, respectively. On day 7, the average count of probiotic species containing potassium chloride mixture and 2% yeast extract was higher than other groups. The average count of the probiotic group containing 1% yeast extract was higher by 0.25 logarithmic cycles compared to the probiotic group. However, the difference between the two groups was not significant (p>0.05). On day 14, the count of the probiotic group containing 2% yeast extract was higher than other groups, but no significant difference was observed between this group and the probiotic group containing 1% yeast extract (p>0.05). The average probiotic count of both groups containing yeast extract was significantly higher than the probiotic group without the mixture of potassium chloride and yeast extract (p<0.05). On day 21, the average count of *B. coagulans* increased with increasing yeast extract concentration, with a significant difference between all groups (p<0.05). On day 28, a similar trend was observed, with no significant difference between the average count of the probiotic group and the probiotic group containing a mixture of potassium chloride and 1% yeast extract (p>0.05). No significant difference was observed between the probiotic group containing a mixture of potassium chloride and 1% yeast extract and the probiotic group containing a mixture of potassium chloride and 2% yeast extract (p>0.05), but the difference in average count between the probiotic group containing 2% yeast extract and the probiotic group without yeast extract was significant (p<0.05). In general, adding yeast extract increased the viability of probiotic species during storage time under refrigerator temperature, as counted on the last day of the study. The average count of the probiotic group containing a mixture of potassium chloride and 2% yeast extract was higher by 0.2 and 0.42 logarithmic cycles compared to the probiotic group containing a mixture of potassium chloride and 1% yeast extract and the probiotic group without yeast extract, respectively.

3.1. Chemical analysis

The results of investigating the effect of the combined use of *B. coagulans* bacteria and a mixture of potassium chloride and yeast extract on the pH of vegetable sausage are shown in Table 3. In the control group, the pH level on days 1 and 7 was calculated as 5.83 and 5.81, respectively, with no significant difference (p>0.05). The pH gradually increased to 5.92 and 5.85 on days 14 and 21, respectively.

Table 3. The effect of the mixture of potassium chloride and yeast extract on the pH level in vegetable sausage during 28 days of storage at 4°C.

Treatment	Day				
	1	7	14	21	28
Control	5.83 ± 0.25 ^{Aa*}	5.81 ± 0.06 ^{Aa}	5.92 ± 0.04 ^{Ba}	5.85 ± 0.04 ^{Ba}	5.75 ± 0.02 ^{Ca}
Probiotic	5.83 ± 0.25 ^{Aa}	5.9 ± 0.03 ^{Aa}	5.88 ± 0.05 ^{Aa}	5.81 ± 0.03 ^{Bab}	5.75 ± 0.03 ^{Ca}
Probiotic + sodium chloride + 1% yeast extract	5.81±0.25 ^{Aab}	5.82 ± 0.03 ^{Aa}	5.8 7 ± 0.03 ^{Aa}	5.83± 0.03 ^{Aab}	5.7 3± 0.03 ^{Ba}
Probiotic + sodium chloride + 2% yeast extract	5.77 ± 0.02 ^{Aa}	5.82 ± 0.02 ^{Aa}	5.84 ± 0.02 ^{Ba}	5.76 ± 0.03 ^{Cb}	5.7 ± 0.02 ^{Da}

*The mean (± standard deviation) with common capital letters in each line indicates no significant difference in (p>0.05). The mean (± standard deviation) with common lowercase letters in each column indicates no significant difference (p>0.05).

The results reported on days 14 and 21 were not significantly different from each other (p>0.05), but the increase in pH on day 14 compared to day 7 was significant (p<0.05). On day 28, the average pH of the studied group decreased to 5.75, which was significantly different from the results of day 21 (p<0.05). For the probiotic group with the usual sodium chloride content, the average pH on day 1 was 5.83, similar to the control group. Although the average pH increased and decreased on days 7 and 14, no significant difference was observed between the calculated average results (p>0.05). On day 21, compared to day 14, the calculated average pH decreased, with a significant difference between averages (p>0.05). On day 28, the calculated average pH decreased to reach 5.75, with a significant difference from the previous calculation day ($p<0.05$). For the probiotic group containing a mixture of potassium chloride and 1% yeast extract, the average calculation made on day 1 was equal to 5.81. Despite its increase with days 7 and 14 results and its reduction on day 21, no significant difference was observed between calculated average values and previous calculation days ($p>0.05$). On day 28, calculated average values decreased to reach 5.73, with a significant difference from the previous calculation day ($p<0.05$). For the studied group of probiotics containing a mixture of potassium chloride and 2% yeast extract, the trend of pH changes during four weeks of study was similar to previous groups. It started from 5.77 on day one and increased to reach 5.82 on day seven, but no significant difference was observed between averages ($p>0.05$). On day fourteen, the calculated average pH increased to 5.84, with a significant difference from the previous day ($p<0.05$). On days twenty-one and twenty-eight, average results decreased to 5.76 and 5.7, respectively, with significant differences from previous calculation days ($p<0.05$). Regarding the average pH of the studied groups during 4 weeks of storage in refrigerator temperature conditions, on day 1, the pH of the probiotic groups containing a mixture of chloride and potassium and yeast extract was not significantly different from each other, but there was a significant difference with the control and probiotic groups. The addition of yeast extract caused a decrease in pH ($p< 0.05$). In general, the addition of probiotics did not change the pH, but the addition of yeast extract to the treatments caused a significant decrease in pH in the studied groups. There was no significant difference between the studied treatments of control, probiotic, and probiotic containing potassium chloride mixture and 1% yeast extract. On days 7, 14, and 28, although the treatments containing yeast extract had a lower average pH, no significant difference was observed between the calculated average pH of the treatments ($p>0.05$). On day 21 of the investigation, the trend of decreasing pH with the addition of yeast extract prevailed, and the difference between average pH results on days 1 and 28 was significant, but other samples did not have a significant difference in average pH results ($p>0.05$).

3.2. Sensory evaluation

The results of applying the probiotic species *B. coagulans* and the mixture of potassium chloride and yeast extract on the sensory characteristics of vegetable sausage are shown in Fig. 2. Regarding overall acceptance by the 32 participating evaluators, the probiotic group containing a mixture of potassium chloride and 2% yeast extract obtained the highest rank with an average rank of 2.12, followed by the control group with a rank of 2.19. The probiotic group containing a mixture of potassium chloride and 1% yeast extract and the probiotic group were ranked third and fourth in terms of overall acceptance. In general, the difference in evaluation rating of the probiotic group with other groups was significant, and the addition of probiotic species caused a drop in the overall acceptance evaluation score. The lack of significant difference between the control and probiotic groups with 1 and 2% yeast extract indicates the effect of yeast extract in improving the evaluation rating of probiotic herbal sausage ($p<0.05$). There was no significant difference between the average evaluation of the probiotic group containing a mixture of potassium chloride and 1% yeast extract with the probiotic treatment containing 2% yeast extract and this treatment with the control and probiotic groups ($p>0.05$).

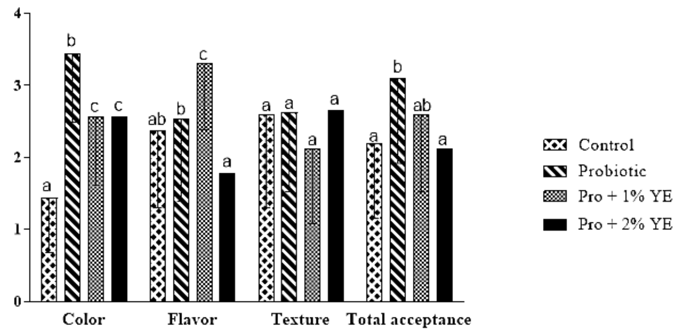

Fig. 1. The effect of the potassium chloride and yeast extract mixture on the sensory characteristics of vegetable sausage during 28 days of storage at 4°C. The mean (± standard deviation) with common lowercase letters in each column indicates no significant difference ($p>0.05$).

However, the difference in average rating of probiotic samples containing a mixture of potassium chloride and 1% with other treatments was significant ($p<0.05$), indicating the effect of 2% yeast extract concentration in improving the taste of probiotic herbal sausage. Regarding sensory index color evaluation, the control group took first place with an average rating of 1.44, followed by probiotic groups containing a mixture of potassium chloride and 2% yeast extract, probiotics containing a mixture of potassium chloride and 1% yeast extract, and finally probiotic group respectively ranked second to fourth. No significant difference was observed in average evaluation ranks between probiotic groups containing 1 and 2% yeast extract ($p>0.05$). Still, the difference in average rank between control and probiotic groups was significant with each other and other groups, indicating the effect of probiotics on the loss of color sensory properties in evaluation, which is compensated by adding yeast extract ($p<0.05$). Regarding sensory evaluation index taste, the probiotic group containing 2% yeast extract received the highest rank from evaluators with a score of 1.78, followed by control, probiotic, and probiotic containing a mixture of potassium chloride and 1% yeast extract groups, ranked second to fourth with ranks of 2.37, 2.53, and 3.31 respectively. No significant difference was observed between the ranking results of the control group and the probiotic group containing 2% yeast or the control group and probiotic group ($p>0.05$). However, the difference in average rating between the probiotic group containing a mixture of 1% yeast extract with other samples was significant, indicating the effect of using 2% yeast extract in improving the taste of vegetable sausage ($p<0.05$). However, the amount of 1% brain extract causes product color to fall. Regarding the sensory evaluation texture of vegetable sausage samples, no significant difference was observed between the studied groups ($p>0.05$). The addition of probiotic species caused a decrease in tissue evaluation scores, but the use of 1% yeast extracts improved tissue. Increasing the amount of yeast extract to 2% caused a decrease in product texture evaluation scores.

4. Discussion

4.1. Survival of probiotics

This study investigated the survival of the probiotic bacterium *B. coagulans* in vegetable sausage containing a mixture of potassium chloride and yeast extract during 4 weeks of storage in the refrigerator. The results showed that adding yeast extract increased the viability of the probiotic species during storage under refrigerator temperature. On the last day of counting, the average count of the probiotic group containing a mixture of potassium chloride and 2% yeast extract was higher by 0.2 and 0.42 logarithmic cycles compared to the probiotic group containing a mixture of potassium chloride and 1% yeast extract and the probiotic group without yeast extract, respectively. These results are consistent with previous studies investigating the survival rates of *B. coagulans* in various food products. For example, Jafari et al. (19) investigated the viability of *B. coagulans* ATCC 31284 during processing and storage in the refrigerator and using different antioxidant compounds. They found a decrease of 3 to 4 logarithmic cycles in the count of probiotic species added to Farsh that occurred after processing and cooking. The count rate decreased significantly after applying thermal shock. However, the count of probiotic species during product processing and storage in the refrigerator was higher than 10^6 CFU/g (19). Ganjoori et al. (20) investigated the survival of *B. coagulans* in enriched bulk bread. They found that the usual baking process caused a decrease of 2 logarithmic cycles in the count of probiotic species, reaching 10^6 CFU/g. After one week of storage in the refrigerator, an average of 10^5 cells per gram of bread survived. By increasing the concentration of inoculated probiotic species in the ready-to-bake dough, the final limit of probiotic species can be reached in this widely used product after baking and until the last day of maintenance at an acceptable level (20). Hashemi et al. (21) investigated survival rates of *B. coagulans* and Lactobacillus acidophilus in low-fat probiotic and synbiotic ice creams after freezing and during 90 days of storage at -18°C. They found that survival rates of *B. coagulans* were higher than Lactobacillus acidophilus after the freezing process and at the end of ice cream storage time. In another study conducted by Jafari et al. (22), the effect of different and common home cooking methods on the viability of Bacillus probiotics in heated sausages was investigated. They found that there was no significant decrease in the number of *B. coagulans* spores in 40% cooked red meat sausages when boiled in water or microwave, but deep cooking in oil caused a significant decrease in number of *B. coagulans* probiotic species for samples with 40, 55, and 70% red meat sausages. However, for 55% of chicken meat sausage, no significant difference was observed between frying and microwave methods. This indicates the effect of product formulation on the survival rate of *B. coagulans* and the type of thermal processing due to the intensity and speed of heat transfer to the internal parts of the sausage. These results suggest that the usual cooking process can cause several logarithmic cycles to decrease the number of *B. coagulans* spores, but adding yeast extract can increase viability during storage time under refrigerator temperature. The probiotic bacterium *B. coagulans* can produce spores, which gives it suitable heat resistance for use in the enrichment of cooked products such as meat and meat analog products, as well as flour products such as bread and pasta. In this study, adding yeast extract increased the viability of the probiotic species during storage under refrigerator temperature. On the last day of maintenance, the average count of the probiotic group containing a mixture of potassium chloride and 2% yeast extract was higher compared to the probiotic group containing a mixture of potassium chloride and 1% yeast extract and the probiotic group without yeast extract. Few studies have been conducted on yeast extract's prebiotic ability to improve probiotic species' viability in food products. In a study by Hekmat et al. (23), the growth and survival rate of *Lactobacillus reuteri* RC-14 and *Lactobacillus rhamnus* GR-1 in yogurt as a beneficial food along with yeast extract prebiotics and inulin were investigated. In treatment containing 0.33% yeast extract, after 1 day of storage, counts of *L. reuteri* and *L. rhamnus* were equal to 8×10^5 and 4×10^7 CFU/ml, respectively. After one month of storage in refrigerator conditions, both probiotic bacteria were higher than non-probiotic treatments, with the highest bacterial count reported in treatment containing 0.33% yeast extract and 0.4% inulin. Al-Manhal and Niameh (24) investigated the effect of using mannan extract of *Saccharomyces cerevisiae* as a prebiotic in yogurt made from buffalo milk. They found that increasing the percentage of yeast extract to 2.5% increased the viability of Bifidobacterium and *L. acidophilus* starters, indicating the prebiotic role of yeast extract in improving the viability of probiotic bacteria. Overall, these results suggest that the addition of yeast extract can increase the viability of probiotic species during storage time under refrigerator temperature.

4.2. pH analysis

The results of the present study showed that the addition of yeast extract caused a decrease in the pH of the studied groups during 4 weeks of storage in refrigerator temperature conditions. The trend of pH changes in the investigated groups during storage in the refrigerator was an increase until day 14 and then a decrease, which can be related to the activity of lactic acid bacteria in the natural flora of the product and their use of available carbohydrate sources as an energy source, leading to the production of lactic acid and other aromatic compounds that increase the acidity of product and decrease its pH during the storage period. Although no significant difference was observed between measurements made on day 28, in groups containing potassium chloride mixture and yeast extract, the decrease in pH was lower than in the control sample, which increased with the concentration of extract. The pH of the group containing potassium chloride mixture and 2% yeast extract on day 28 was equal to the lowest amount (5.7). According to studies conducted by Champagneh et al. (25) and Glabert et al. (26), replacing part of sodium chloride with potassium chloride does not change the pH of the product, but adding 1 to 2% yeast extract caused a significant decrease in pH. This decrease is probably related to carbohydrate compounds in yeast extract that are easily available for fermentation by lactic acid bacteria in product flora. In general, reducing the pH of the product is important as it can help improve environmental conditions by inhibiting the growth of spoilage and pathogenic microorganisms, ultimately leading to the production of a product with longer shelf life. This can be considered a strong point in national distribution and supply chain, along with improving product health assurance and ultimately strengthening product marketing.

4.3. Sensory evaluation

A comprehensive and accurate assessment of a new product's properties, sensory characteristics, and formulation plays a key role in its market development. The results of this study showed that in the evaluation of the sensory index of overall acceptance, adding probiotic species to vegetable sausage caused a decrease in the score. However, this score drop was compensated by adding yeast extract, but increasing the concentration to 2% improved the overall acceptance score compared to the control sample. As a result, the probiotic samples containing a mixture of potassium chloride and 2% yeast extract received the highest rating. In general, adding *B. coagulans* probiotic species to meat products and many other food groups can cause a decrease in overall acceptance. This is due to the loss of tissue caused by the probiotic species' glycolysis, lipolysis, and proteolysis and the production of metabolites with an unpleasant taste (27). The proteolysis pattern in processing and shelf life of meat or meat analog probiotics (vegetable sausage) is influenced by various factors, including formulation and type of probiotic species used (28). Regarding the sensory evaluation index related to the product's taste, the probiotic group containing a mixture of potassium chloride and 2% yeast extract received the highest rating. Although there was no significant difference between the evaluation results of the control and probiotic samples, the control group obtained higher scores than the probiotic species, indicating the negative effect of probiotic bacteria on the taste of the product under investigation and its compensation by added yeast extract. In general, loss of taste in a meat product or probiotic meat analog (vegetable sausage) is greatly influenced by the amount of fat and protein in formulation and processes of proteolysis, lipolysis, and glycolysis carried out by added probiotic, leading to the production of compounds and metabolites with anti-taste that are preserved over time. *B. coagulans* bacterium affects protein and peptides in food by producing extracellular proteases. Aldehydic, alcoholic, and acidic compounds produced as a result of the breakdown of amino acids leucine, valine, phenylalanine, methionine, etc., have a very low threshold limit, indicating its significant effect on loss of taste in food (31). Adding yeast extract at the rate of 2% to probiotic vegetable sausage can improve taste from the consumer's point of view due to the formation of a wide variety of volatile compounds from the fermentation of carbohydrates and the catabolism of amino acids. Replacing sodium chloride with potassium chloride can cause a bitter and metallic taste, which is a limiting factor regarding this method for reducing sodium content in the product (33). Glabrit et al.'s research in 2003 showed that replacing 40 to 50% of sodium chloride with potassium chloride increases the bitter taste in fermented sausages and sausages (26). The evaluation results related to the probiotic vegetable sausage containing a mixture of potassium chloride and 1% yeast extract (getting the lowest evaluation rating) confirm this issue and are consistent with the results of the mentioned studies. Although in the study conducted by Guardia and his colleagues in 2008, no significant difference was observed in the taste of fermented sausages with 50% replacement of potassium chloride with sodium chloride, the use of yeast extract due to the creation of volatile compounds can largely compensate for the bitter taste and improve the taste of sausages and sausages containing substituted potassium chloride (16). According to the results of the present research, increasing the concentration of yeast extract by 2% with increasing flavor-intensifying properties due to the presence of glutamic acid, inosinate, and guanylate compounds, and increasing the amount of volatile flavor-producing compounds, in addition to compensating for unpleasant flavors caused by the activity of probiotic species and alternative potassium chloride led to a higher evaluation rank than the control sample. No significant difference was observed between the studied groups regarding the sensory evaluation index related to the product's texture. In 2011, Radolovic et al. (34) investigated sensory characteristics of probiotic fermented sausage containing *Lactobacillus helveticus and Bifidobacterium longum* species during 40 days of storage related to ripening. Their results regarding tissue produced on days 14 and 28 showed that investigated cases regarding species containing *Bifidobacterium longum* starter had lower evaluation scores than the control group and the group containing *Lactobacillus helveticus* starter. Another study found that using starter probiotic species *Lactobacillus plantarum* and *Pediococcus damnusus* in low-fat fermented sausage causes stiffness and dryness due to the release of gas from fermentation and the activity of microorganisms (35). In a study conducted by Erkila et al. (36) related to sausage tissue, it was also found that the sensory evaluation score related to the probiotic sample's consistency and texture was slightly lower than the control sample. According to the results of the present study, the probiotic samples' evaluation score increased with the addition of 1% yeast extract, although the difference in average scores between groups was insignificant. The study by Campagnol et al. (37) also found that adding a mixture of potassium chloride and yeast extract improved the evaluation score related to the product's texture. However, the average difference between the investigated groups was insignificant. In this regard, the results of the present study are relatively consistent with those of previous studies. Regarding the evaluation index related to the color of the product, the control group took first place with an average score of 1.44, followed by probiotic groups containing a mixture of potassium chloride and 2% yeast extract, probiotics containing a mixture of potassium chloride and 1% yeast extract, and 1 and 2% yeast extract. However, the difference in average rating between the control and probiotic groups with each other and other groups was significant, indicating the effect of probiotics on the loss of color sensory properties in the evaluation and its compensation by adding yeast extract. The reduction of the average evaluation score related to the product's color in probiotic samples can be due to the increase in the level of oxidation of fats and the loss of added pigments caused by the activity of probiotic species. In a study by Hossein, Razavi, and Imam Juma (38), they investigated the physicochemical and sensory characteristics of beneficial fermented sausage with reduced fat, using *Lactobacillus casei* and *paracasei* species to produce probiotic groups. The results of their study indicated a decrease in sensory characteristics associated with groups containing probiotic species compared to the control sample, which is consistent with the results of the present study. As mentioned earlier, adding yeast extract compensated for the decrease in sensory evaluation score related to the product's color. In a study by Vidal et al. (17) on sensory properties associated with adding yeast extract to salted meat, adding 5% yeast extract improved sensory index related to product color. The average evaluation rating of the group containing 50% substituted potassium chloride was equal to 5.85, while the evaluation score of the group containing 5% added yeast extract was significantly higher at 6.43. The results of their study in this field are consistent with the present study.

5. Conclusion

This study investigated the viability of *Bacillus coagulans* probiotic bacteria in reduced-salt probiotic sausage containing a mixture of potassium chloride and yeast extract and its physicochemical and sensory characteristics. The results showed that the addition of yeast extract improved the survival of *B. coagulans* probiotic bacteria in the group containing yeast extract compared to the probiotic group without yeast extract. The group containing yeast extract also decreased pH during storage time in the refrigerator and improved sensory properties related to taste, color, and overall acceptance of vegetable sausage. Therefore, the simultaneous use of potassium chloride mixture, yeast extract, and probiotics in analog products similar to meat is an effective measure to improve consumption patterns. These products can serve as suitable substitutes for food items considered harmful to health, such as meat-based sausage. However, using the right concentration in the product according to the sensory evaluation results is important.

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finally probiotic group ranked second to fourth. No significant difference was observed in average ratings between probiotic groups

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