



## Investigating the effect of black seed extract on the physicochemical, sensory and anti-mold properties of lactic cheese

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### ABSTRACT

The general objectives are to investigate the anti-fungal properties of black seed extract, and to investigate the physicochemical, sensory and anti-fungal properties of black seed extract. In this research, in order to study the inhibitory effect of black seed ethanol extract at levels of 0, 1, 2, and 3% on the growth of lactic cheese molds, 10 cm slices were prepared after making lactic cheese and the extracts were Different concentrations were added. It was placed in a greenhouse at a temperature of 26 degrees Celsius for 3 weeks. Then, on days 1, 15, 30, 45 and 60, the sensory, physicochemical and texture characteristics were checked. The results showed that the pH of the samples increased compared to the control sample; Examining the amount of moisture in the samples during storage first shows a decrease and then an increase. According to these results, the use of black seed extract does not have a significant effect on the dry matter. Examining the cheese texture results showed that the use of black seed extract does not have a significant effect on consistency. The results obtained to check the hardness of the cheese texture were the highest and the lowest hardness of the sample with black seed extract on day 60 and the control sample on day 1, respectively. Also, the highest elasticity was found in the sample with black seeds on day 60 and the lowest in the control sample on day one. The amount of phenolic compounds in cheese samples with black seed extract is significantly higher than the control sample. Also, the results of the evaluation of sensory characteristics showed that according to the obtained averages, the control sample has the highest average and the third, second and first samples have obtained other averages respectively. In the examination of the microbial properties of cheese, mold and yeast counts were negative in all samples.

## 1- Introduction

Milk and its products are one of the most important parts of human diet in different parts of the world. The production of dairy products has a long history, but it is a sensitive, risky and vulnerable business. Whole food milk has all the necessary elements in a balanced way. Obviously from the attack Microbes are not immune and if there is not enough care in its preparation, storage and distribution due to contamination The microbial spoils quickly, and its consumption will not only compensate for the lack of nutrients in the body Rather, it may lead to infection or food poisoning [1].

From the point of view of the World Food Organization and the World Health Organization, cheese is a fresh or ripe solid or semi-solid product made from milk, skimmed milk or cream coagulated by the action of Rennet enzyme or other appropriate coagulants, then separating the whey. can be This product has high-quality protein and is very rich in essential amino acids. Also an important source of minerals, especially calcium (That), on (Zn), phosphorus (P) and magnesium (MB) Is. Cheese is known as a very useful food, so that its use has a direct and significant effect on health. Cheese is a food rich in complete protein that can replace meat. Cheeses as sources of bioactive peptides<sup>1</sup> are considered, cheese hydrolysis occurs during the cheese making stage, the resulting peptides act as precursors of bioactive molecules [2].

Throughout history, plants have had a special relationship with humans. The general tendency to use herbal medicines and natural products in general has been increasing in recent years. Black seed plant with scientific name *Nigella sativa*. It has a rich medical and religious history and has been used by ancient Egyptians and Greek doctors to treat headaches, asthma, allergies, toothaches and strengthen the immune system, etc. [3]. Black seed is a one-year plant from the alala family, which is the natural flora of southern Europe, northern Africa, and southwestern Asia. The seeds of this plant contain oil, protein, alkaloid (such as niglycine and nigelidine), quinones (such as thymoquinone), saponin and volatile essential

oil. The pharmacological and biological effects of black seed and thymoquinone, as one of the components of black seed, include things such as antioxidant, anti-ischemic, anti-inflammatory and analgesic, anti-epileptic and anti-cough effects. chemical mixture The Black SeedsIt has shown its high nutritional potential, especially as a source of protein and fat. The seeds of this plant contain 30-40% oil, 20% protein, 5.7% moisture, 0.5-15% essential oil. Black seed has unsaturated fatty acids, carbohydrates, vitamins C, E and mineral elements of calcium, iron and potassium, plant sterols and alkaloids, phospholipids and carotene according to pharmacological effects. The Black Seedsand its consumption in traditional medicine, and on the other hand, its antioxidant and antimicrobial properties can be used as a food. pragmatistused [4]. Samati and Noghani (2016) investigated the effect of savory powder on total forms, mold and yeast in local cheese. The findings of this research showed that by increasing the percentage of Khuzestan savory powder in the cheese samples, the total number of mold and yeast, titratable acidity, general acceptability andpH It significantly decreased and increased, respectively. Alimohammadzadeh et al. (2019) Antimicrobial effect of alcoholic extract and essential oil of black cumin (*Bunium persica* Boiss) They studied some bacteria and molds in lactic cheese [5,6].

Due to the fact that the use of black seeds in the production of cheese causes an undesirable color and appearance, as well as the lack of uniformity of the plant in the texture of the product, and alsoDue to having more phenolic compounds, and less fragrance than essential oils that have terpenoid compoundsIt is preferred to use black seed extract (blue, hydroalcoholic, etc.). According to the mentioned materials, the purpose of this research is to investigate the properties of black seed hydroalcoholic extract in juniper production and to investigate the physicochemical, sensory and anti-fungal properties of black seed extract.

## 2- Materials and methods

### 2-1- Materials

All materials needed for the project including cheese curd and starter (*Lactobacillus bulgaricus* and *Lactobacillus lactis*) were obtained from Binalud company. Black seed was also prepared from the Attari level of Neishabur city. Also, the chemicals used in this research include; Sulfuric acid, ethanol, sodium hydroxide, amylic alcohol, phenolphthalein, etc., which were purchased from reputable representatives of Merck.

### 2-2- Methods

#### 2-2-1- Black seed extract extraction

The seeds of the black seed plant were purchased from local and reliable centers for selling medicinal plants in Neyshabur. In the oven device (made by the company MEMMERT) was completely dried at a temperature of 50 °C and was crushed by an electric shredder (model A11From company YOU Germany) were completely powdered mechanically. To prepare the ethanolic extract of the plant, 100 grams of the dried powder of the plant seeds were added to 400 milliliters of pure ethanol and the resulting solution was gently stirred for one hour using a sterile magnetic stirrer until it was completely mixed.

Then the obtained solution was stirred again after it was placed at room temperature for 24 hours, and after passing through a cloth filter and collecting the remaining waste materials, the solution containing the extract was also passed through filter paper. Then the solvent (ethanol) was removed by evaporation in a rotary evaporator. The obtained extract was placed in a sterile glass container under freezing conditions.

#### 2-2-2- Preparation of cheese

First, the chemical composition of raw milk used in cheese preparation, such as the amount of acidity, fat, solid matter without fat, density and pH. It was determined according to the standard that is given in Table 1. After pasteurization at 65 degrees for 30 minutes and cooling to 35 to 38 degrees Celsius, whey in the amount of 0.07 grams for 5 kilos of milk and starter at a ratio of 1% weight/weight after dissolving in distilled water Sterile was added to the milk and kept at that temperature for 60 minutes until a cheese crust is formed. Formed cut into small pieces until the whey comes out. After the water was removed from the rind, black seed hydroalcoholic extract was added to the cheese rind in concentrations of 0, 1, 2, and 3%. The scab was pressed and then cut into certain sizes and kept in salt water for a day and then transferred to jars containing 8% salt water [7].

Table 1. Chemical compositions of raw milk used in cheese preparation

parameter	amount
acidity	14/4
pH	6/73
fat	3/4
density	1/030
water	0
SNF	8/3

#### 2-2-3- adding extract to black seed

In this research, in order to study the inhibitory effect of black seed hydroalcoholic extract at

levels of 0, 1, 2 and 3% on the growth of lactic cheese molds, and after preparing lactic cheese, 10 cm slices were prepared and the extracts were added with different concentrations. It

was kept in a greenhouse at a temperature of 26 degrees Celsius for 3 weeks. Sampling of cheeses started from the day of pressing the rinds (first day) and was repeated on days 0, 15, 30, 45 and 60. All the tests are done during the ripening period and sensory evaluation of the cheese will be done on the 30th and 60th day of ripening [8].

### 2-3- Research tests

#### 2-3-1- measurement pH

measurement pH by directly inserting the electrode of the device pH meter into the homogenized cheese texture. It should be noted that the device pH meter before measuring pH Calibrated [9].

#### 2-3-2- Measuring humidity

The moisture content of the cheese samples was measured by weight method by drying 2 grams of cheese in a glass plate that had previously been brought to a constant weight, at a temperature of 102 degrees Celsius in an oven until the constant weight of the sample was reached. ]10[.

#### 2-3-3- Tissue analysis

Test TPA and cheese hardness using the device. Analyzer Texture It was done at 10°C. for the test TPA Using a 6 mm cylindrical probe at speeds/mm 0.5 to a depth of 4 mm was done. For the pressure test, first the cut wire probe is connected to the device and after Cheese slices were placed in square shapes so that their dimensions are slightly larger than the width of the cutting probe ]11[.

#### 2-3-4- Measurement of the total amount of phenolic compounds

To measure total phenolic compounds, Folin Ciocalto's reagent was used. After taking the extract 0.5 ml Ethanol extract was mixed with 5 ml Folin Ciocalto and then 4 milliliter One molar sodium carbonate was added. After adding the solutions, the color of the phenolic extract became slightly dehydrated, because the phenolic compounds in the extract are subjected to oxidation and reduction reactions with phosphomolybdic acid in Folin Ciocalto

reagent, and due to the alkalinity of the reagent medium, the reaction occurs spontaneously. goes. Blue is the color of molybdenum. For the control, instead of dry extract, distilled water was used and then folin ciocalto and sodium carbonate were added. This solution was used to zero the spectrophotometer. The above solution was placed in the dark for 15 minutes. Absorption of samples at a wavelength of 765 nm by Spectrophotometer read]12[.

#### 2-3-5- Evaluation of sensory characteristics of cheese samples

Sensory test of cheese after 1, 15, 30, 45, 60 days of ripening was done by 12 panelists using 5-point hedonic method. The features measured by the panelists include: color, taste, smell, texture and overall score, with scores of 5 (excellent), 4 (good), 3 (moderate), 2 (weak) and 1 (unsuitable) [13]. .

#### 2-3-6- Evaluation of microbial characteristics

Under sterile conditions, 20 grams of cheese sample was completely homogenized and dissolved and homogenized in 180 ml of sterile sodium citrate. Then the required successive dilutions were prepared depending on the tested microbial culture. All cultures were performed in triplicate ]14[.

#### 2-3-7- Counting mold and yeast

The surface culture method was used to count mold and yeast population. Environment YGC It was used at a temperature of 25 degrees Celsius for 72 hours [15].

#### 2-3-8- Statistical evaluation

Data collected in a completely randomized design in 3 replications using computer software SPSS were analyzed. Comparison of treatment means using Duncan's multiple range

test at the 5% probability level and graphs using software Excel was drawn

### 3- Discussion and results

#### 3-1- Measurement pH

Level pH It is necessary to maintain the quality of cheese during the storage period. Diagram (3) of the effect of adding different ratios of black seeds on pH Shows examples. The highest amount for the sample with black seed extract was on day 1 and 15, and the lowest amount for the control and treatment sample was on day 60. During the period of reaching the rate pH decreases, and the reason for this phenomenon

can be justified by the set of metabolic processes of lactic acid bacteria and the production of organic acids, especially lactic acid [16].

Also, increasing the activity of Renth enzyme and reducing the negative charge of casein micelles decreases the amount pH It is possible [17]. The results of this research with the results of Dezirani et al. (1400) that The effect of using edible coating of basil seed mucilage along with different levels of black cumin extract on the quality properties and shelf life of lactic cheese. checked it was matched. Also, Awad and his colleagues (2014) in the study of processed cheese samples treated with cod puree showed that simultaneously with the passage of time pH Samples are significantly reduced.

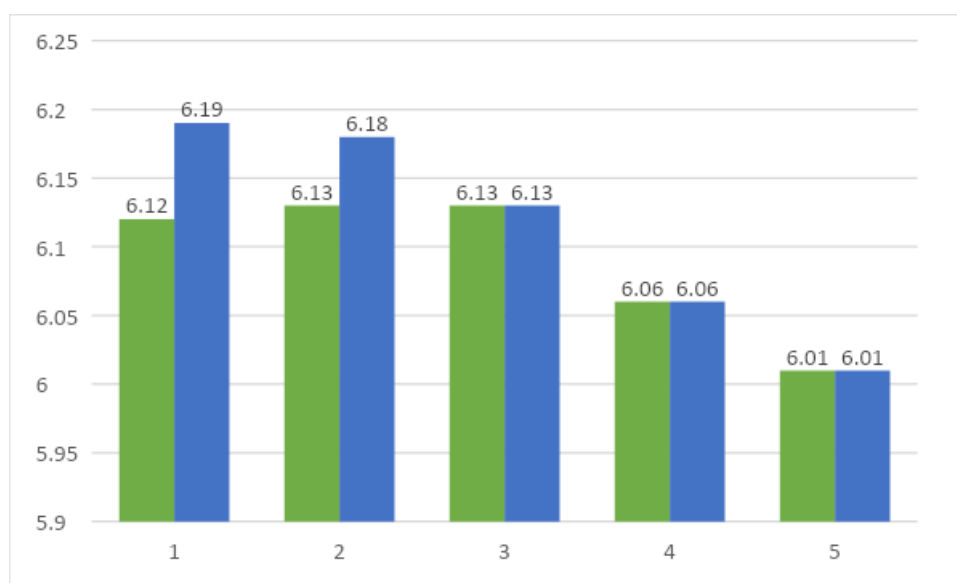


figure 1. The effect of different proportions of black seed extract on the pH of the samples.

#### 2-3- Measurement of humidity

According to the results obtained in the diagram (2), the effect of adding different proportions of black seed on the moisture level of the samples during storage first decreases and then increases. The lowest and highest moisture levels for the control sample were on the 45th and 30th days. With the passage of time, due to continuous migration of water to the surrounding environment, the amount of water decreases. Finally, there is no significant difference in the moisture content of the samples. The results of a research showed that by examining the performance characteristics

of pizza cheeses, Mozzarella and Process during the storage period, they showed that the moisture content does not differ significantly with the passage of time compared to the control sample [18].

Also, Arianfar et al. (2016) by investigating the antioxidant and antimicrobial effect of thyme essential oil on mozzarella cheese stored at refrigerator temperature reached similar results in terms of moisture content. These results are in agreement with the results of Papa and Malatova (2005) regarding the decrease in the moisture content of mozzarella cheese during the storage period. They stated that during the first 20 days of ripening, the moisture content decreased at a high rate, which they mentioned

as the reason for the reduction of hydrogen in casein during this time period.

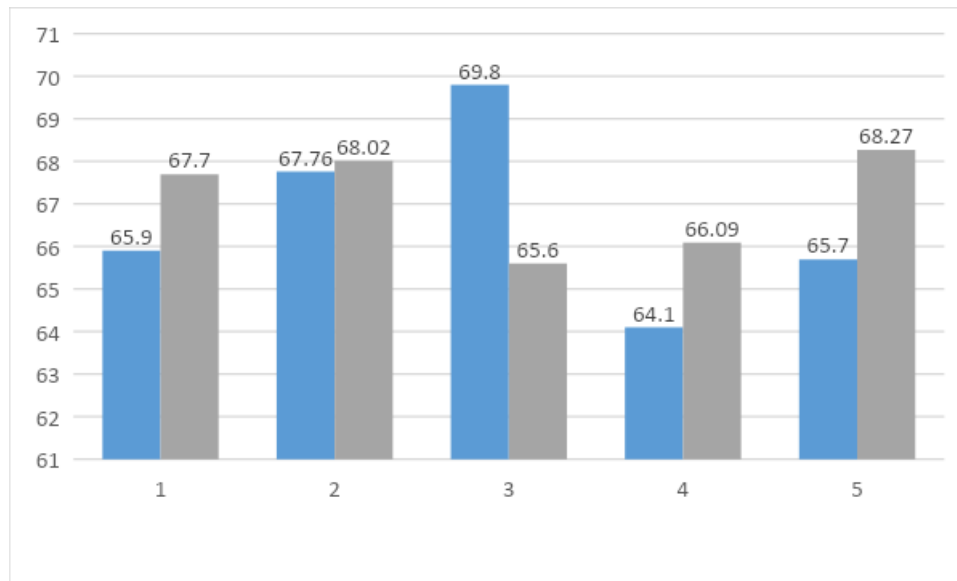


figure 2. The effect of different proportions of black seed extract on the moisture content of the samples.

### 3-3- Checking the amount of dry matter

The amount of dry matter of the samples can be seen in graph (3). According to these results, the effect of adding different proportions of black seeds on the amount of dry matter of the samples has no significant effect. The lowest and the highest amount of dry matter for the control sample is on days 30 and 45. Lashkari

and Momeni Sarvestani (2019) by investigating the effect of black cumin essential oil on the physicochemical, microbial and sensory characteristics of ultra-refined feta cheese reached similar results in terms of dry matter. Also, Veridi et al. (2018) investigated the effect of adding pomegranate juice on the physical-chemical, microbial and sensory characteristics of feta cheese without dehydration and showed that the dry matter of the samples does not show significant changes during storage.

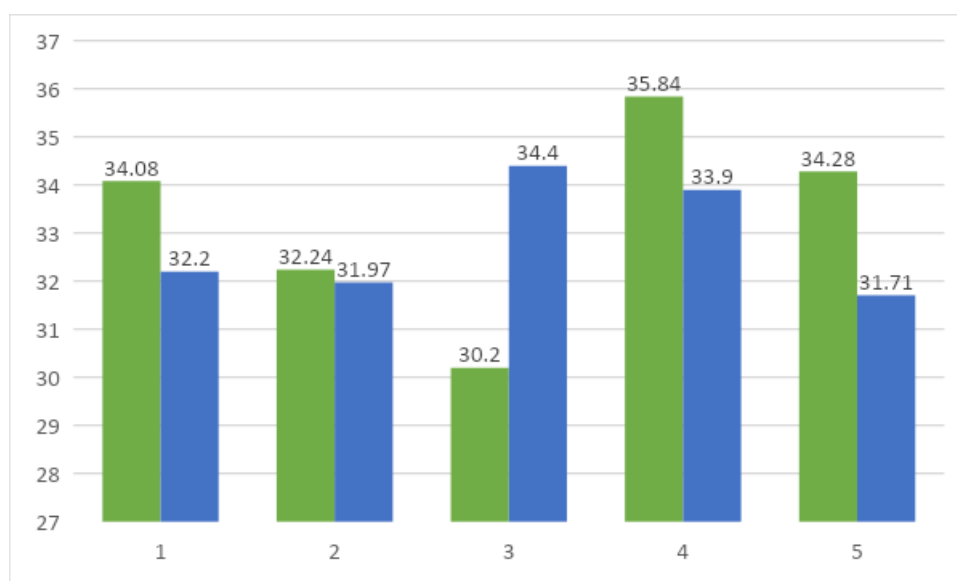


figure 3. The effect of different proportions of black seed extract on the amount of dry matter of the samples

### 3-4- Tissue analysis

The feeling caused by touching foods is often considered one of their important characteristics. Aspects to consider include durability(Firmness), gentleness(Softness), cheerfulness(Juiciness), Chewable, fibrous, sandy state(Grittiness), oily state(Oiliness) And finally, crispiness(Tenderness). Conceptual What we still don't have a good definition for is texture. Texture is related to adhesion, stretchability and other physical properties of food. The texture properties of food are divided into geometric, mechanical properties (particle size, shape and position) and properties related to moisture and fat content. The primary indicators in The mechanical properties are cohesion hardness, viscosity, stretchability, and adhesion. Secondary indicators include crispness, chewing properties, and gummy properties. Different types of texture have been identified based on compression, tensile strength and hardness(Toughness) are graded. The degree of hardness of a food item can be found by cutting and separating its different parts, without losing the shape of each separated section. Scales for measuring hardness, crispness, chewiness, stickiness and viscosity of the texture have been introduced with suitable standards. These scales are used in sensory evaluation of the texture [19].

According to graph (4), the effect of adding different ratios of black seeds on the continuity is not significant. The continuity of the samples over time can be attributed to the increase in proteolysis of the produced cheese protein, which leads to an increase in the percentage of small peptides. The highest degree of adherence was found in the cheese sample with black seed extract on day one, and the lowest degree of adherence was found in the control sample during different periods.

Also, the results obtained to check the hardness of the texture are given in graph (5). The effect

of adding different proportions of black seed on tissue hardness shows that the highest and lowest tissue hardness was related to the sample with black seed extract on day 60 and the control sample on day one, respectively. The hardness of the cheese texture is attributed to the calcium in the milk. Calcium in milk binds to the casein matrix. pH reduces the negative charge of casein, which neutralizes the protein interference– Protein gets between the micelles and increases the accumulation of micelles. As a result, it increases the hardness of the cheese texture [20].

The results of the investigation of the effect of adding different ratios of black seeds on the elastic state of the samples are given in graph (6). The highest elasticity was found in the sample with black seeds on day 60 and the lowest in the control sample on day one. During the storage period, due to the formation of the calcium network, the hardness of the tissue increases and the elasticity decreases. Also, because the proteins of the cheese tissue remain healthy during storage, it cannot form many protein chains, therefore the structure becomes fragile [21].

Mohammadifar et al. (2013) by examining the textural and sensory characteristics of ultra-refined white cheese produced with the protease of Paneerbad plant (*Vitania coagulans*) compared to mushroom rennet, reached similar results in terms of texture consistency. Luo and his colleagues (2011) in research that evaluated the effect of adding different proportions of calcium on the texture and microstructural biochemical characteristics of fresh cheese containing different percentages of isolated sesame protein as a partial substitute for milk proteins concluded that after the period Ripening increases the hardness of cheese, which was consistent with the results of this research. Also, Yurqanlou and Ghibi (2018) reached similar results in examining the texture of yogurt containing dill extract.

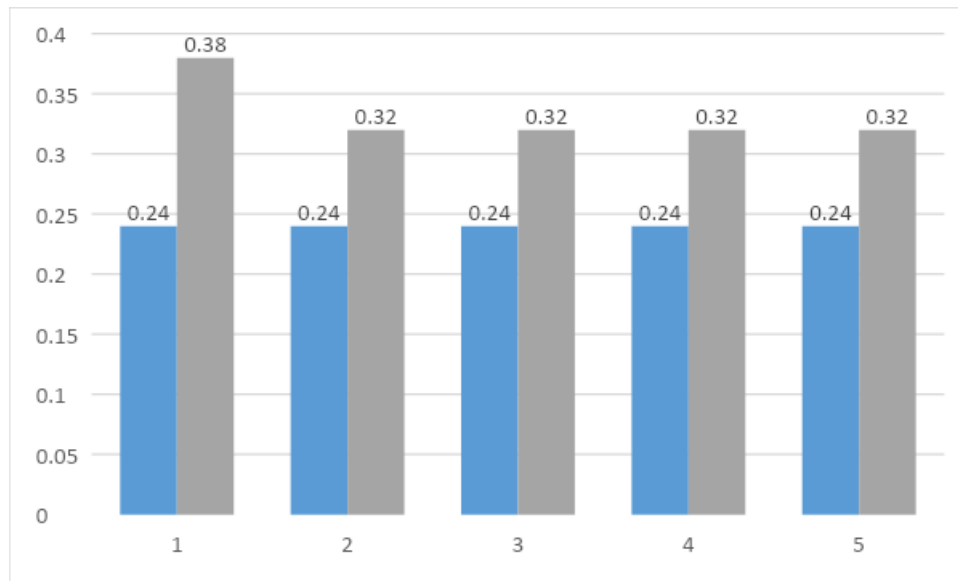


figure 4. The effect of different proportions of black seed extract on the consistency of the samples.

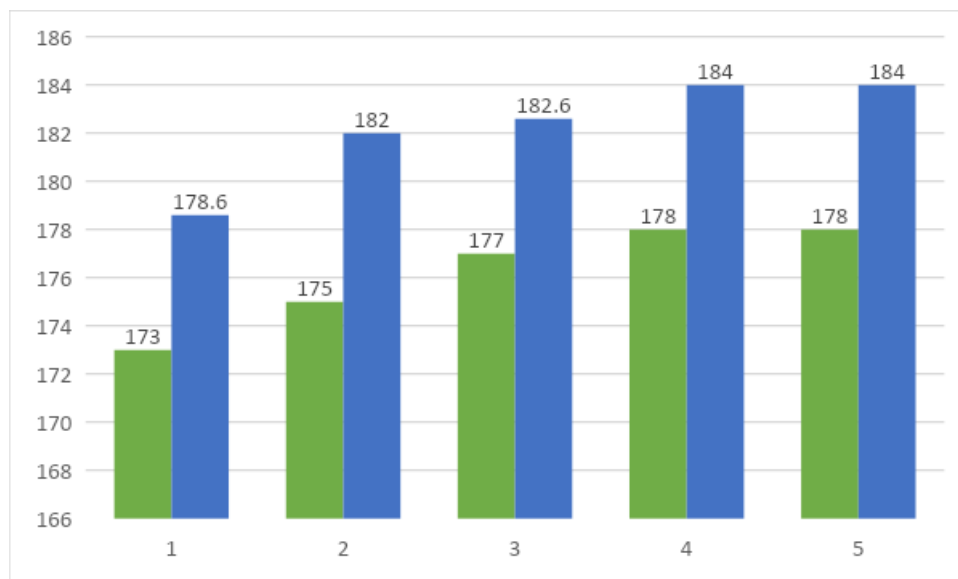


figure 5. The effect of different proportions of black seed extract on the hardness of the samples.



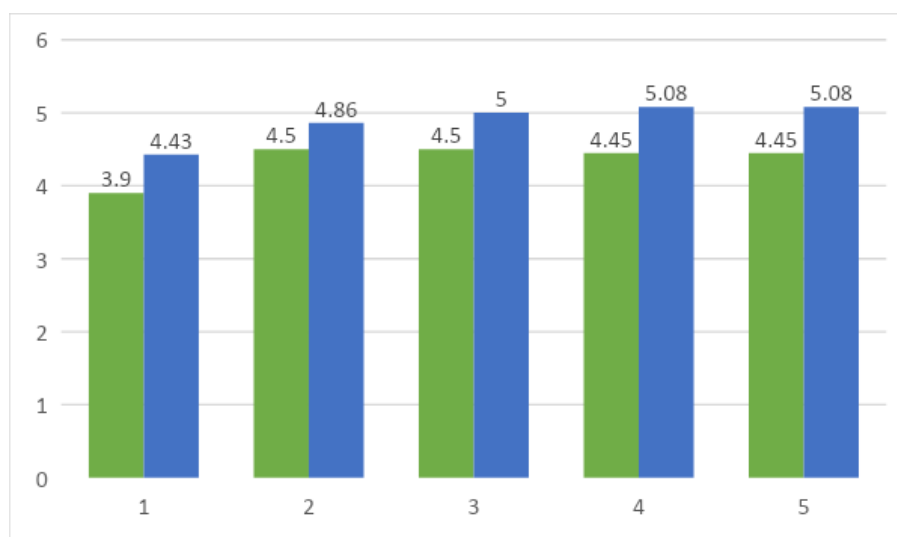


figure 6. The effect of different proportions of black pepper extract on the elastic state of the samples.

### 5-3- Measuring the total amount of phenolic compounds

According to the results of graph (7), the effect of adding different ratios of black seeds on the amount of phenolic compounds in cheese samples is significant. With the increase of the storage period, the amount of these compounds has increased. So that the highest amount of phenolic compounds in the sample has black seed extract and on day 60. The presence of phenolic compounds in the control sample is due to the presence of polyphenols in milk, which is mainly caused by animal feeding. The presence of phenolic compounds (flavonoids and flavonols) in high amounts in black seeds and the presence of these compounds in the product has increased the amount of phenolic compounds in the product [22].

Phenolic compounds are plant secondary metabolites with multiple biological effects

such as antioxidant activity and antibacterial activity. According to the results of Mortezaei et al. (2012); Black seed extract has  $3.05 \pm 57.33$  milligrams per milliliter is equivalent to gallic acid of phenolic compounds [23]. These compounds exert their antioxidant effects by inhibiting free radicals or limiting their production cycle. Also, the effective compounds of plants, in addition to their effect on free radicals, delay or prevent oxidation by reacting with metals and other compounds that trigger the oxidation process, as well as suppressing oxygen active compounds.]18[. Rabbanifard et al. (2022), investigating the effects of essential oil and extract of black cumin (Bunium peach) on the microbial and chemical properties of traditional lactic cheese showed that the alcoholic extract of black cumin has higher phenolic compounds than black cumin essential oil in the final composition of lactic cheese.

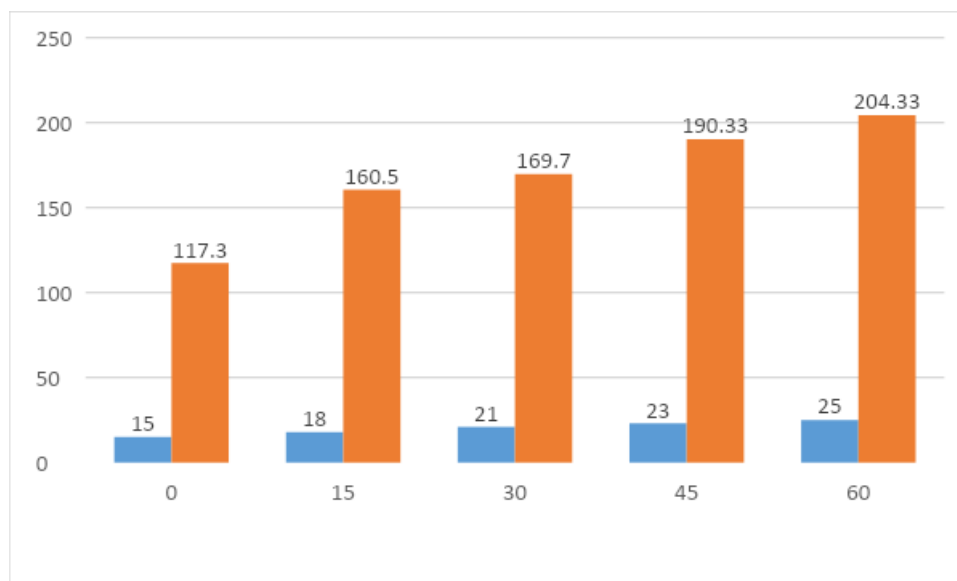


figure 7. Effect of different proportions of black seed extract on the phenolic compounds of the samples.

### 3-6- Examination of sensory characteristics

Sensory characteristics (taste, taste, smell, texture and overall acceptance) were performed according to the national standard number 4938, so that during the 60-day storage period of the cheeses on the 15th, 30th, and 60th days to evaluate the external appearance (shape, surface crust), internal appearance (color) , holes and apertures), texture and consistency (bending and

squeezing, chewing), aroma and smell, taste (bitterness, saltiness, spiciness, general desirability of taste) and their overall acceptance and evaluation were provided to the sensory evaluator group and for each The above characteristics were scored on a 5-point scale (1 score for the most unfavorable and 5 for the most favorable sample).The results of sensory characteristics are given in table 2. According to the obtained averages, the control sample has the highest average and the third, second and first samples have obtained the other averages respectively..

Table 2. Average sensory property data of the sample.

Number	Average
The witness	4/14
The first example	3/69
The second example	3/92
The third example	3/96

### 7-3- Examining microbial characteristics

Mold and yeast counts were negative in all samples. The reason for this can be an indicator of proper pasteurization conditions, hygienic

production process and black seeds without contamination. The main cause of taste loss and unfavorable physicochemical changes in cheese with black seed extract is related to the growth of mold and yeast during storage. The results of microbial tests showed that none of

the treatments were contaminated with coliforms and they were destroyed during pasteurization. Also, the total count was negative in all samples. Coliforms need a temperature of 7 to 44 degrees Celsius for proper activity, and their presence in food indicates fecal contamination. If the environmental conditions are out of this range and hygiene tips are maintained during the storage of samples, coliforms cannot grow anymore]24[. The absence of bacteria in the overall count apart from heating during the process and compliance with hygienic conditions during production can be due to the presence of compounds such as lignans (sesamol, sesaminol and other lignans) in black seed extract because these lignans have antioxidant and antimicrobial activity. and... are also[15]. As mentioned before, the presence of these compounds, with the property of inhibiting free radicals or limiting their production cycle, causes the oxidation process to start, as well as quenching the active oxygenated compounds, delaying or preventing oxidation. In addition, due to the synergistic effect and antimicrobial effect of black seed extract along with pH Low and acids produced during the storage period, as well as the decrease in moisture percentage compared to the control sample, can be one of the reasons for the negative growth of bacteria during the storage period [25].

#### 4- Summary

In this research, the effect of using black seed extract in lactic cheese was investigated in different periods. pH The samples show an increase compared to the control sample; The highest amount for the sample with black seed extract was on day 1 and 15, and the lowest amount for the control and treatment sample was on day 60. It also decreases during the ripening period. Checking the amount of moisture in the samples during storage first shows a decrease and then an increase. The lowest and highest moisture levels for the control sample were on the 45th and 30th days. According to these results, the use of black seed extract does not have a significant effect on dry matter. The lowest and highest amount of dry matter for the control sample is on days 30 and 45. Examining the cheese texture results

showed that the use of black seed extract has no significant effect on consistency. The continuity of the samples over time can be attributed to the increase in proteolysis of the produced cheese protein, which leads to an increase in the percentage of small peptides. The highest degree of adhesion was found in the cheese sample with black seed extract on day one, and the lowest degree of adhesion was found in the control sample during different periods. The results obtained to check the hardness of the cheese texture were the highest and the lowest hardness of the sample with black seed extract on day 60 and the control sample on day 1, respectively. Also, the highest elasticity was found in the sample with black seed on day 60 and the lowest in the control sample on day one. The amount of phenolic compounds in cheese samples with black seed extract is significantly higher than the control sample. Also, with the increase in the storage period, the amount of these compounds has increased. So that the highest amount of phenolic compounds in the sample has black seed extract and on day 60. Also, the results of examining the sensory characteristics showed that according to the obtained averages, the control sample has the highest average, and the third, second, and first samples have obtained other averages, respectively. In the examination of the microbial characteristics of cheese, mold and yeast counts were negative in all samples.

#### 5- Resources

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## بررسی تاثیر عصاره سیاه دانه بر ویژگی‌های فیزیکوشیمیایی، حسی و ضد کپکی پنیر لاکتیکی

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اطلاعات مقاله	چکیده
تاریخ های مقاله : تاریخ دریافت: ۱۴۰۲/۳/۲۲ تاریخ پذیرش: ۱۴۰۳/۲/۴	در این پژوهش جهت مطالعه اثر ممانعت کنندگی عصاره اتانولی سیاه دانه بر رشد کپک در پنیر لاکتیکی سطوح ۰، ۱، ۲ و ۳ درصد به پنیر تهیه و پس از تولید پنیر لاکتیکی، برش های ۱۰ سانتی متری از آن آماده و عصاره ها با غلظت های مختلف افزوده شد. نمونه ها به مدت ۳ هفته در دمای ۲۶ درجه سانتی گراد گرمخانه گذاری شد. سپس در روزهای ۱، ۱۵، ۳۰، ۴۵ و ۶۰ ویژگی های فیزیکوشیمیایی، بافتی و حسی نمونه ها بررسی شد. نتایج نشان داد pH نمونه ها در مقایسه با نمونه شاهد افزایش نشان یافت. میزان رطوبت در نمونه ها طی نگهداری ابتدا کاهش و سپس افزایش داشت. نتایج مربوط به سختی بافت پنیر نشان داد که بیشترین و کمترین سختی بافت به ترتیب مربوط به نمونه دارای عصاره سیاه دانه و در روز ۶۰ و نمونه شاهد در روز اول بود. هم چنین بیشترین میزان حالت ارتجاعی در نمونه دارای سیاه دانه و در روز ۶۰ و کمترین مقدار در نمونه شاهد و در روز اول بود. میزان ترکیبات فنولی در نمونه های پنیر دارای عصاره سیاه دانه نسبت به نمونه شاهد تفاوت معنی داری داشت. بررسی ویژگی های میکروبی نشان داد که شمارش کپک و مخمر در تمامی نمونه ها منفی بود.
کلمات کلیدی: عصاره، سیاه دانه، پنیر لاکتیکی	
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