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Effect of addition of microcapsules produced with Cress Seed Gum and Acacia Senegal Gum containing thymol on the quality and shelf life of functional yogurt during storage in the refrigerator

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ABSTRACT

Yogurt is a popular dairy product among dairy products and is prone to spoilage due to the growth of bacteria and other microorganisms. In this research produced active microcapsules based on thymol containing Acacia senegal gum and cress seed gum (0.1%, 0.25%, and 0.5% w/v) as a natural preservative and in different concentrations were added to the food model (yogurt). The physicochemical, antimicrobial, Viscosity, and sensory properties of the yogurt samples were evaluated during the 60 days of storage in the refrigerator. The results showed that with increasing concentration, thymol, capsule efficiency, and antimicrobial activity of microcapsules against the studied bacteria, including Escherichia coli, Salmonella enteritidis, and Aspergillus niger, increased significantly. However, the produced microcapsules did not have a significant antimicrobial effect on Lactobacillus delbrueckii. The evaluation results showed that at the end of the storage period, samples containing microcapsules in higher thymol concentration received higher scores regarding texture, taste, and overall acceptance. Based on the results obtained from the counting, microbial, syneresis rate, sensory evaluations, and moldiness tests, yogurt samples containing microcapsules based on cress seed gum (0.5%) and thymol (0.5%) had the longest storage time and the highest quality compared to other samples until the end of the storage period. Findings show that the use of 5% cress seed gum as a natural preservative in the structure of thymol-based active microcapsules is recommended for keeping yogurt at refrigerator temperature.

Keywords: Acacia senegal gum; Microcapsule; Thymol; Cress seed gum; Yogurt

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1. Introduction

Yogurt is a fermented milk product produced by lactic acid bacteria. Two starters are used in yogurt production (Streptococcus thermophilus and Lactobacillus bulgaricus). Yogurt, often considered one of the most popular fermented dairy products in the world, has a wide range of health benefits in addition to basic nutrition. In general, due to its nutritional profile, yogurt is a rich food and a high calcium source that provides significant amounts of calcium in a bioavailable form. Yogurt is a good carrier for the delivery of probiotics, and when consumed, it brings unparalleled health benefits (El-Abbadi et al., 2014).

The increase in consumers' awareness regarding the nutritional and sensory aspects of yogurt has caused producers to pay more attention to its appearance, aroma, and texture characteristics during production, so the achievement of texture characteristics has been considered in numerous researches. One of the limitations of yogurt product storage is its moldiness and fat oxidation during the storage period, which limits the shelf life and consumption of yogurt (Khalifah et al., 2019). Due to its lower pH than other dairy products, yogurt has a longer shelf life than these products and can remain in the refrigerator for about 7 to 10 days without microbial spoilage. Surface spoilage occurs in yogurt by acid-resistant molds and yeasts. The main cause of yogurt spoilage is mesophilic yeasts, which affect the quality characteristics of yogurt. Mesophilic yeasts increase the final product's microbial load, reducing the activity of starter

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microorganisms and even destroying them in yogurt. Also, the findings of a study conducted on the physicochemical, sensory, and microbial quality characteristics of the yogurt product showed that among 100 yogurt samples, no type of infection with *Staphylococcus aureus* and *Salmonella subspecies* was observed. However, some samples were declared positive for mesophilic bacteria, coliform, *Escherichia coli* and fungus (Mirza Alizadeh et al., 2014).

Some preservatives are harmful to humans and may be classified as carcinogens. Among the natural compounds that can be used as food preservatives are plant essential oils and phenolic compounds. These compounds have antibacterial, antifungal, antioxidant, and anticancer properties (Jafarpour et al., 2022). It is a natural volatile monoterpenoid phenol, the main active ingredient of the oil extracted from the species (Thymus vulgaris L.) commonly known as thyme. It is a versatile molecule with a wide range of applications. Thymol is recognized as a safe additive. This substance has low solubility in water and is soluble only in temperatures above 52 °C. But it dissolves well in fat (Escobar et al., 2020). Acacia senegal gum (AG) is an edible, dry exudation of the Acacia AG trees. It is non-viscous and soluble in fiber. Acacia senegal gum is a mixture of polysaccharides and glycoproteins (GPs) that give it adhesive properties. AG is used as an emulsifier and a thickening agent in chewing gum and other sweets (Chikami et al., 1997). Cress seed gum with the scientific name (Lipidium satium) belongs to the Crucifera family and is generally called "garden cress". When the seeds are soaked in water, they absorb moisture quickly and create a sticky and tasteless substance. The seeds have a high molecular weight and many mucilage chemicals. The major part of the extract is a carbohydrate and mannose primary sugar (Prajapati et al., 2014).

Microencapsulation is an advanced food processing technology that allows any compound to be encapsulated inside a specific material, creating a small sphere with a diameter ranging from 1 μ m to several μ m. Micro-encapsulation is done to protect sensitive ingredients and hence ensure their safe delivery. Capsules can be polymeric or non-polymeric materials such as cellulose, ethylene glycol, and gelatin. Various techniques are used for microencapsulation, Fluidized bed coating, spray cooling, spray drying, extrusion, and coaxing. Microencapsulation leads to the protection of different food components or functional components against different processing conditions. In addition, it enhances the sensory quality by masking unpleasant taste, aroma, and flavor (Choudhury et al., 2021).

Several researches have been conducted on the use of nanoemulsions of bioactive compounds in yogurt, for example Salama et al. (2022) during the production of probiotic yogurt containing nanoemulsions of different plant essential oils stated that bacterial strains L. helveticus, L. acidophilus, and B. bifidum lactis were able to survive in the presence of different nanoemulsions with a bacterial count >10⁶ cfu/ml. Various rheological and physicochemical evaluations showed that nanoemulsions did not affect the general characteristics of yogurt samples during the 15day storage period and overall sensory evaluation showed that all samples had very good sensory scores. Shi et al. (2024) in investigating the effect of nanoemulsions containing resveratrol on yogurt samples, stated that the physicochemical and functional properties of enriched yogurt samples were improved. The results showed that the addition of resveratrol emulsion decreased the hardness of yogurt and increased the water holding capacity. The stability of resveratrol added in the emulsion form was significantly higher than that of the free form. In vitro digestion showed that microencapsulation effectively and consistently improved the bioavailability of resveratrol.

Considering the mentioned explanations, the use of the microencapsulation method of the functional active compound of thymol in the biocompatible coating material such as cress seed gum and Acacia senegal gum in reducing oxidation and increasing the shelf life of a high-consumption product such as yogurt can open the way for the industry and ensure the public health of the society (Chikami et al., 1997).

2. Material and Methods

2.1. Materials

Acacia senegal gum (AG) and cress seed gum were purchased from the Seed and Plant Improvement Institute (Karaj, Iran). Potato dextrose agar, MRS Agar (De Man–Rogosa–Sharpe agar), Sodium hydroxide, and other chemicals from Merck (Germany), and thymol from Sigma-Aldrich (USA).

2.2. Methods

2.2.1. Cress seed gum extraction

Cress seed gum extraction About 45 g of seeds were washed 3 times with alcohol (ethanol), approximately 2 or 3 times the volumetric weight of the seeds, on the stirrer at a speed of 1000 rpm for 15 min to remove all foreign matter such as dust, dirt, and stones. After removing ethanol from the seeds by filtration followed by evaporation in an oven at 70°C, they were steeped in distilled water (water to seed 10:1) at 35°C for 8 h. Then water is added to 30 times the weight of the seed, and its separation is done with a rod paddle blender (Rondo-2500, KA702, France) at 5000 rpm for 10 min to remove gum from the seed; Separation of the gum from the swollen seeds was achieved by filtration with cheesecloth (Khazaei et al., 2014).

2.2.2. Preparation of active microcapsules containing thymol

Acacia senegal gum powder and cress seed gum powder in different concentrations of 0.1 and 0.5% dissolved in water, and each was stirred separately using a stirrer at 1100 rpm for 1 h at room temperature and then kept overnight at 4 °C to ensure the dissolution of biopolymers. Then, thymol was dissolved in 1 cc of oil to the desired amount and added to the desired amount after dissolving. Then, we use tween's appropriate amount of 1% to form an emulsion. Oil-in-water emulsion was prepared by spreading the concentrations of 0.1%, 0.25%, and 0.5% of thymol in the desired solutions. 1% of calcium chloride was added to the prepared solutions to stabilize the microcapsules. Then, to form microcapsules, the solutions prepared from the desired concentration with different percentages were homogenized for 2 min using a ULTRA-TURRAX high-speed homogenizer at 11000 rpm. The desired microcapsules were prepared using a spray dryer (Niu et al., 2016). Treatments for microcapsule formation are shown in Table 1.

Т9

T10

T11

T12

Treatment	Acacia senegal gum (% w/v)	Cress seed gum (% w/v)	Thymol (% w/v)
T0	0	0	0
T1	0.1	-	0.5
T2	0.1	-	0.1
T3	0.1	-	0.25
T4	-	0.1	0.5
T5	-	0.1	0.1
T6	-	0.1	0.25
T7	0.5	-	0.5
T8	0.5	-	0.1

0.5

0.5

0.5

Table 1. Treatments for microcapsule formation.

2.2.3. Tests performed on microcapsules

0.5

2.2.3.1. Evaluation of the morphology of microcapsules

The prepared samples were evaluated using an optical microscope and the formation and non-formation of microcapsules were checked (Chen et al., 2006).

2.2.3.2. Checking the size of microcapsules

Checking the size of microcapsules was done with a Malvern zetasizer (zen 3600) made in the USA (Chen et al., 2006).

2.2.4 Preparation of functional yogurt containing active microcapsules

For the preparation of functional yogurt, microcapsules according to Table 2, at 1% were added to yogurt, and mixing was done gently. Then, the samples were transferred to the incubator to set the yogurt and then in the refrigerator at 0 to 4 °C to be used on the test days (Khorshidi et al., 2021).

Table 2. Treatments of yogurt samples containing microencapsulated thymol

Treatment	tment Acacia senegal Cress seed gum gum (% w/v) (% w/v)		Thymol (% w/v)
TY0	0	0	0
TY1	0.1	-	0.1
TY2	0.25	-	0.25
TY3	0.5	-	0.5
TY4	-	0.5	0.1
TY5	-	0.5	0.25
TY6	0.5	0.5	0.5

2.2.5. The tests performed on functional yogurt containing active microcapsules

2.2.5.1. pH

The equation: First, the pH meter was calibrated using pH 4 and pH 7 buffer solutions. For this purpose, 25 ml of buffer

solution was poured into a 50 ml beaker, and the electrode was placed in it. Then 10 g of yogurt sample was poured into the beaker and made uniformly with a glass stirrer. The pH of yogurt samples was measured with the help of an electrode (AOAC,2012).

2.2.5.2. Acidity

To measure the acidity, 20 g of the sample was weighed and dissolved in water, and after putting all its contents into a 250 ml volumetric flask, it was brought to volume and filtered using a strainer. 0.5 ml of phenolphthalein reagent was added to it and titrated. This process was continued until a pale pink color appeared, which remained stable for at least 5 S (AOAC, 2012).

2.2.5.3. Viscosity

0.25

0.5

0.1

0.25

The viscosity of yogurt samples was measured using a Brookfield viscometer made in Germany. In this test, the LV4 spindle was used. All tests were performed at 20 °C and under the same conditions so the samples' viscosity was read at 30 rpm and after 15 S of spindle rotation (Eghdaie Amiri et al. 2010).

2.2.5.4. Enumeration of yogurt starter culture microorganisms

Yogurt starter culture microorganisms were counted using the colony counting method. In this way, decimal dilutions of the test were poured into the plate, and then the specific culture media below were added to them. Each dilution was cultured in two plates. MRS agar culture medium with acidic pH was used to count *Lactobacillus bulgaricus*. Plates cultured in anaerobic conditions were kept in an incubator at 37 °C for 72 h. Counted colonies were confirmed by specific tests (Green& Ibe, 1987).

2.2.5.5. Syneresis

To measure the syneresis of yogurts, 25 g of sample was weighed on Whatman No. 41 filter paper and placed on the funnel. The amount of water removed from the funnel after 120 min at a temperature of 4 °C was expressed as water release, and the percentage of synergism was calculated (Amatayakul et al., 2006).

2.2.5.6. Sensory evaluation

Sensory evaluation was done by 30 trained evaluators. Scoring was done on a 5-point hedonic scale from very bad (1) to very good (5). The samples were evaluated for overall acceptance (Eghdaie Amiri et al., 2010). To check the appearance of moldiness, the yogurt samples were stored in the refrigerator for 60 days, and on days 1, 15, 30, and 60, the samples were checked for the appearance of mold (Buehler et al., 2018).

2.3. Statistical Analysis

The average of each parameter was calculated by one-way analysis of variance (ANOVA) and analyzed using SPSS 16.0 software. Differences between treatments were expressed in Duncan's multiple tests at the 95% level (P<0.05). The graphs were drawn using EXCEL 2013 software.

3. Results and Discussion

At first, there were 12 treatments for the formation of active microcapsules containing thymol, under the light microscope after preparation was observed that the number of treatments with a concentration of 10% of Acacia senegal gum and 0.1% of cress seed gum as coating with different concentrations of thymol (0.1%, 0.25% and 0.5%) did not form microcapsules. In this order, only the remaining 6 treatments with a concentration of 0.5% of Acacia senegal gum and a concentration of 0.5% of cress seed gum as coating with different concentration of 0.5% of Acacia senegal gum and a concentration of 0.5% of cress seed gum as coating with different concentrations of thymol (0.1%, 0.25%, and 0.5%) could form microcapsules and were tested. Fig. 1 shows the light microscope of the formation of microcapsules and the lack of formation of microcapsules.



Fig. 1. Optical microscope image of microcapsule.

3.1. Particle size of microcapsules

Table 3 shows the average particle size of microcapsules. Based on the average size results, the highest size of microcapsules belonged to the T5 and T6 treatments and the lowest to the T1 treatment; with increasing thymol concentration in the treatments, the particle size increased. Also, the types had a significant effect on the size of the microcapsules (P<0.05); due to the increase in the concentration of the microencapsulated compound, the size of the particles increased. This research was consistent with the results of Okonogi and Riangjanapatee in 2015, who investigated nanostructured lipid carriers containing lycopene and observed that with increasing lycopene content, particle size and particle coupling rate increased significantly.

Table 3. Mean and standard deviation of microcapsules particle size (μm)

Treatment	particle size
T1	125 ± 15
T2	163 ± 11
T3	175 ± 13
T4	195 ± 14
Т5	215 ± 22
T6	223 ± 19

3.2. Results of the tests performed on functional yogurt containing active microcapsules

3.2.1. pH and acidity

The pH and acidity of the yogurt samples (Tables 4-5), respectively decreased and increased from the 10th to the 30th day of the storage period. The increase in the activity of lactic acid bacteria in yogurt led to acid production and a decrease in pH until day 30 of the storage period. From the 30th day onwards, the pH increased with the reduction of the microbial load in the samples, the consumption of nutrients by the bacteria, and the production of nitrogenous compounds from the proteins in yogurt. Research conducted by Shafie (2018) showed that the pH of yogurt samples containing microencapsulated Lactobacillus plantarum during 8 weeks of storage time was significantly higher than that of free bacteria and control samples (p<0.05). The results of the research of Alireza loo et al. (2015) showed that in all of the samples during the storage time, pH decreased, and the reason for this phenomenon can be more related to the production of lactic acid by lactic acid bacteria, which can produce 4 molecules of lactic acid from 1 molecule of lactose. frying.

3.2.2. Viscosity

The results showed that the samples with microcapsules based on cress seed gum and Acacia senegal gum containing thymol had a higher viscosity than the control sample (Table 6). this increase in viscosity is because the hydrocolloids increase the viscosity by blocking the free water in the sample. Also, with the increase in storage time, the viscosity of the samples increased, which is caused by the rearrangement of proteins and protein binding changes. In addition, the viscosity of the samples increased with increasing time because of syneresis and evaporation of moisture. Haji Ghafarloo et al. (2020) showed that with increasing amounts of Acacia senegal gum, the viscosity of buttermilk samples increased and phase separation decreased significantly (p<0.05) and phase separation in buttermilk enriched with Acacia senegal gum was slower than the control sample and the samples containing the ginger extract.

3.2.3. Microbial population of starter culture microorganisms

The results show that the yogurt starter culture microorganisms had an upward trend until the 30th day and a downward trend until the 60th day, a significant increase and decrease (Table 7). It is probably because the nutrients needed by the microorganisms are high in the beginning and until the 30th day, the nutrients are available to the microorganisms, but after that, with the decrease of the nutrients, a downward trend was created in the starter microorganisms. This trend is consistent with the results of Mehraban Sangatash et al. (2023), who stated that the number of starter culture microorganisms in yogurt increased and decreased during 28 days of cold storage. In the last few days, the number of these microorganisms reached the lowest level. It can also be said that thymol-containing microcapsules had no inhibitory effect on the starters of production yogurt samples. Several reports have been presented regarding the survival of starter culture microorganisms in vogurt, each with different results. For example, Shirvani et al. 2020

Treatments	Day 1	Day 15	Day 30	Day 45	Day 60
TY0	3.76±0.01 Ab	3.76±0.02 ^{Ba}	3.49±0.01 Dd	3.63±0.01 ^{Ccd}	3.76±0.01 Ac
TY1	3.74±0.02 Ab	3.71±0.06 Aa	3.54±0.01 Bd	3.45±0.01 Bf	3.43±0.01 Bg
TY2	3.76±0.01 Ab	3.76±0.01 Aa	3.49±0.01 Dd	3.56±0.01 ^{Ce}	3.56±0.01 Bf
TY3	3.76±0.01 Ab	3.75±0.03 Aa	3.56±0.01 ^{Cc}	3.63±0.01 Bc	3.69±0.01 Ad
TY4	3.77±0.01 Ab	3.75±0.01 Ba	3.56±0.01 Dbc	3.61±0.01 ^{Cd}	3.63±0.01 ^{Ce}
TY5	3.83±0.02 ^{Ba}	3.76±0.03 ^{Ca}	3.57±0.01 Dab	3.76±0.01 Ba	4.02±0.01 Aa
TY6	3.75±0.01 Bb	3.76±0.02 ^{Ca}	3.53±0.01 Da	3.70±0.02 ^{Bb}	3.87±0.01 Ab

Table 4. Changes in pH values of different yogurt treatments during storage time.

Different small letters indicate a significant difference in the column and different capital letters indicate a significant difference in the row (p<0.05).

Table 5. Changes in the acidity of different yogurt treatments during storage time.

Treatments	Day 1	Day 15	Day 30	Day 45	Day 60
TY0	1.35±0.03 Db	2.06±0.01 Ba	2.31±0.03 Aa	2.02±0.01 Bb	1.75±0.01 Bb
TY1	1.35±0.03 Eab	1.95±0.02 Aa	2.19±0.01 Ac	1.99±0.01 Bab	1.85±0.01 ^{Bb}
TY2	1.35±0.03 Db	1.85±0.01 ^{Cc}	2.29±0.01 Aab	2.06±0.01 Ba	1.83±0.01 Bb
TY3	1.35±0.03 Db	1.82±0.03 Ccd	2.06±0.01 Ad	1.95±0.01 Bc	1.75±0.01 Bb
TY4	1.37±0.03 Dab	1.81±0.02 ^{Cd}	2.06±0.01 Ad	1.95±0.01 Bc	1.82±0.01 ^{Bb}
TY5	1.38±0.03 Eab	1.87±0.02 ^{Cd}	2.19±0.01 Ac	1.95±0.01 Bc	1.70±0.01 Bb
TY6	1.45±0.03 ^{Ea}	1.99±0.01 ^{Cb}	2.26±0.01 Ab	2.02±0.01 Bb	1.76±0.01 Bb

Different small letters indicate a significant difference in the column and different capital letters indicate a significant difference in the row (p<0.05).

Table 6. Changes in viscosity (MPa.s) of different yogurt treatments during storage time.

Treatments	Day 1	Day 15	Day 30	Day 45	Day 60
TY0	4.10±0.20 Ea	9.04±0.21 Dcd	10.13±0.25 ^{Cd}	12.05±0.27 ^{Bd}	13.69±0.28 Ad
TY1	3.28±0.04 Ec	8.90±0.01 Dd	10.68±0.20 Ccd	14.79±0.11 Bc	18.90±0.01 Aa
TY2	4.38±0.00 Da	9.58±0.10 ^{Cab}	9.58±0.11 ^{Ce}	14.24±0.06 Bc	18.90±0.00 Aa
TY3	3.56±0.06 Ebc	7.39±0.12 Df	9.31±0.13 ^{Ce}	11.50±0.10 Bd	13.69±0.20 Ad
TY4	3.83±0.18 Da	9.04±0.13 ^{Cbc}	14.79±0.03 ^{Bb}	16.71±0.50 Aa	17.80±0.49 Ab
TY5	4.38±0.27 ^{Ea}	9.58±0.16 Da	10.95±0.16 ^{Cc}	14.24±0.30 Bc	17.53±0.47 Ab
TY6	4.10±0.20 Ea	9.04±0.21 Dcd	10.13±0.25 ^{Cd}	12.05±0.27 ^{Bd}	13.69±0.28 Ad

Different small letters indicate a significant difference in the column and different capital letters indicate a significant difference in the row (p<0.05).

Table 7. Changes in the microbial population of starter microorganisms of different yogurt treatments during storage time.

Treatments	Day 1	Day 15	Day 30	Day 45	Day 60
TY0	6.21±0.20 Bab	5.57±0.21 Bbc	9.07±0.25 Aa	3.71±0.27 ^{Cd}	3.21±0.28 ^{Cbc}
TY1	6.28±0.04 ^{Ba}	6.92±0.01 Babc	8.00±0.20 Aa	4.14±0.11 ^{Ccd}	2.92±0.01 ^{Cc}
TY2	5.85±0.00 Babc	7.57±0.10 Aa	7.86±0.11 Aa	5.00±0.06 ^{Cab}	4.14±0.00 Dab
TY3	6.00±0.06 ^{Cab}	7.35±0.12 Bab	8.64±0.13 Aa	4.85±0.10 Dbc	2.98±0.20 Ec
TY4	5.57±0.18 ^{Cc}	6.57±0.13 Bbc	8.24±0.03 Aa	5.42±0.50 ^{Ca}	4.14±0.49 Dab
TY5	6.01±0.27 Aab	7.00±0.16 Aabc	7.42±0.16 Aa	4.71±0.30 Abcd	4.57±0.47 Aa
TY6	5.85±0.20 Bb	5.55±0.21 Bc	7.85±0.25 Aa	3.78±0.27 ^{Cd}	3.57±0.28 ^{Cbc}

Different small letters indicate a significant difference in the column and different capital letters indicate a significant difference in the row (p<0.05).

reported a gradual decrease in the number of starter culture microorganisms in yogurt by adding walnut leaf extract until the end of the 15th day. Jalalvand et al. (2022) showed that the viability of probiotic bacteria in heat-treated buttermilk samples decreased significantly with increasing storage time and the amount of cress and Spirulina platensis. However, the effect of these mentioned extracts on the number of starter culture microorganisms is not significant compared to the control sample. In this research, the effect of the thyme plant on the activity of Lactobacillus acidophilus as a probiotic yogurt starter bacterium was carried out. The survival of Lactobacillus acidophilus during yogurt storage at 4 °C was investigated during specific time intervals. The results showed that the number of starter culture microorganisms decreased significantly after 7 days of storage. Also, no significant difference was observed between the control and samples containing different concentrations of thyme essential oil (p<0.05).

3.2.4. Syneresis

In this research (Table 8), during the maintenance period, all treatments' syneresis rates had a statistically significant increase up to the 60th day (p<0.05). However, the syneresis of the samples was less than the control treatment. Syneresis is an undesirable feature that occurs due to the rearrangement of the gel network and an increase in particle connections. Therefore, the network wrinkles and the internal liquid leaks out. According to the research by Behnia et al. (2014) investigated the effect of cress seed gum mucilage on low-fat yogurt's rheological and textural properties. Nowadays, due to the increasing desire to consume low-fat or fat-free products, it is preferred to use fat-free milk to prepare yogurt. The cress plant has many medicinal uses, and since it has been determined that the seeds of this plant contain a large number of mucilaginous compounds, in this research, cress seed gum was used in concentrations of (0.05,

Treatments	Day 1	Day 15	Day 30	Day 45	Day 60
TY0	10.02±0.06 Bab	10.02±0.06 Be	12.43±0.08 Ab	13.51±1.09 Aab	14.86±2.23 Aabc
TY1	9.72±0.39 ^{Ca}	11.08±0.06 Bb	12.97±0.08 Ab	12.70±0.11 Ab	13.24±0.16 Ac
TY2	10.54±0.15 ^{Cab}	10.67±0.08 Bc	12.43±0.96 Ab	12.83±0.78 Ab	14.05±0.24 Ab
TY3	10.02±0.25 Dab	12.29±0.05 ^{Ca}	14.59±0.02 ^{Ba}	14.86±0.06 Aa	15.67±0.86 Aa
TY4	10.02±0.12 ^{Ca}	12.70±0.03 Ba	14.84±0.33 Aa	14.59±0.48 Aab	14.59±0.48 Aab
TY5	10.00±0.42 Bab	10.54±0.06 Bd	12.16±1.03 Ab	11.35±0.46 Ac	12.70±0.16 Ab
TY6	10.13±0.04 Bab	12.97±0.56 Aa	14.32±0.32 Aa	15.13±1.25 Aa	15.40±1.96 Aab

Table 8. Changes in syneresis of yogurt treatments during the storage time.

Different small letters indicate a significant difference in the column and different capital letters indicate a significant difference in the row (p<0.05).

Table 9. Changes in average overall acceptance scores of different yogurt treatments during storage time.

Treatments	Day 1	Day 15	Day 30	Day 45	Day 60
TY0	4.22±0.35 Ab	4.05±0.00 Aa	4.01±0.00 Aa	4.09±0.35 ^{Bb}	-
TY1	5.00±0.00 Aa	4.22±0.35 ^{Ba}	4.01±0.00 Ba	3.21±0.35 ^{Cb}	-
TY2	4.47±0.71 Aab	4.22±0.35 Aa	4.80±1.06 Aa	3.46±0.71 Aab	3.29±0.35 Aa
TY3	4.01±0.00 Abc	4.47±0.71 Aa	3.01±0.00 Aa	4.09±0.35 Bb	-
TY4	4.73±0.35 Aab	4.22±0.35 Aa	4.52±0.71 Aa	4.05±0.00 Aa	2.76±0.35 ^{Ba}
TY5	4.78±0.71 Aab	4.47±0.71 Aa	4.22±0.35 Aa	3.46±0.00 Bb	3.25±0.35 ^{Ba}
TY6	4.73±0.35 Aab	4.22±0.35 Aa	3.80±0.35 Aa	3.29±0.35 Ab	-

Different small letters indicate a significant difference in the column and different capital letters indicate a significant difference in the row (p<0.05).

Table 10. Presence or absence of mold in yogurt samples during the 60-day storage period.

Treatments	Day 1	Day 15	Day 30	Day 45	Day 60
TY0	5±0.0 Aa				
TY1	5±0.0 Aa				
TY2	5±0.0 Aa				
TY3	5±0.0 Aa	5±0.0 Aa	5±0.0 Aa	5±0.0 Aa	1±0.0 Bb
TY4	5±0.0 Aa				
TY5	5±0.0 Aa				
TY6	5±0.0 Aa	5±0.0 Aa	5±0.0 Aa	5±0.0 Aa	1±0.0 Bb

Different small letters indicate a significant difference in the column and different capital letters indicate a significant difference in the row (p<0.05).

0.07, 0.1, and 0.15 percent by weight). The produced yogurt's pH, acidity, hydration percentage, viscosity, and texture (firmness and stickiness) were evaluated during storage (1, 7, 14 and 21 days). By adding to the low-fat yogurt samples, the pH of the samples decreased compared to the control sample. A significant increase in acidity was observed during the storage period. As the concentration increased, the amount of hydration in the samples decreased. The hardness of the samples increased during the storage period. This research showed that cress seed gum hydrocolloid has good potential as a stabilizer in yogurt formulation. Castillo et al. (2006) found that the increase in temperature over time increases the amount of syneresis. In another research conducted by Lucey in 2002, it was found that in acidic gels such as yogurt, the rearrangement of the casein gel network during the storage period is considered the most important in releasing water from the network. Shafie (2018) in the investigation of physicochemical and sensory changes of probiotic yogurt containing free and microencapsulated Lactobacillus plantarum during storage time, stated that the reason for the syneresis in yogurt samples containing alginate capsules is the collapse of the structure and the decrease in the strength of the yogurt gel.

3.2.5. Sensory evaluation

3.2.5.1. Overall acceptability

Dairy products are widely consumed worldwide, so their acceptance by consumers is very important. The characteristics of

dairy products, such as aroma, taste, texture, and color (appearance), interest buyers; therefore, sensory evaluation of these products should be done through global methods. Sensory science has advanced significantly in the past decade and is rapidly evolving to become a key tool for food prediction. Sensory evaluation is a scientific discipline used to understand how humans perceive and respond to different stimuli in food using the five senses of sight, smell, touch, taste, and hearing (Civille and Oftedal, 2012). In this research (Table 9), a scoring or hedonic test was used to evaluate the sensory characteristics of yogurt, with 5 as the highest and 1 as the lowest. The results of the examination of yogurt treatments containing active thymol microcapsules showed that the highest taste score was related to the samples of the first day and the lowest score was related to the samples of the last day. The results related to the color and texture of the yogurt treatments containing active thymol microcapsules showed that the highest score according to the evaluators was related to the samples of the first day, and during the storage time, the scores of the treatments decreased in terms of texture and color, so that the lowest score was related to the treatments of the last day. The results related to the smell test showed that increasing the storage time of yogurt containing active thymol microcapsules did not significantly affect the smell in the yogurt samples, so the smell score of the samples did not change over time on the first and last day. The results of the overall acceptance test showed that with the increase in the storage time, the overall acceptance score decreased, so the highest score was related to the samples of the first day and the lowest score was related to the samples of the last day. Also, there was a trend of flavor changes in cream cheese samples, so with increasing storage time, the taste,

color, and texture acceptability of cream cheese samples decreased (Mahajan et al., 2015).

3.2.5.2. Moldiness

Milk and milk-based products provide a suitable environment for the growth of microorganisms such as yeasts, molds, and a wide range of bacteria. The change in the microbial count is attributed to the degree of contamination and processing temperature in different stages of milk processing. It is accepted that heat treatment of milk, such as pasteurization, enables its safe ingestion of foodborne illness. At the same time, its negligence occurred to a sufficient extent both in the exit of products and in the occurrence of food diseases. As a result, mold spoilage is one of the problems of the milk industry, especially fermented products (Dash et al., 2022). The results (Table 10) show that the yogurt treatments containing thymol-active microcapsules in low rolls and the control treatment had moldiness on the 60th day. Also, thymol at higher concentrations, i.e., 0.5% and 0.25% prevents moldiness during storage. This could be due to the reduction of starter microorganisms in yogurt, which provided the conditions for the growth of cake with the downward trend of microorganisms on the 60th day and also the low concentration of thymol. On the other hand, due to the resistant coating structure of cress seed gum-based microcapsules compared to Acacia senegal gum-based microcapsules, it was better able to preserve the composition of thymol during the storage period and prevent the moldiness of the food samples containing these microcapsules until the 60th day. Amini Bidokhti et al. (2012), In the study of the effect of thyme plant extract and essential oil against Aspergillus niger and Geotrichum candidum and starter bacteria in yogurt, stated that the best inhibitory effect against Aspergillus niger and Geotrichum candidum is related to 0.5% thyme essential oil. Except for the concentration of 1% of thyme essential oil, no inhibitory effect on the starter bacteria was observed in the rest of the concentrations of extract and essential oil of thyme. Also, the samples containing low concentrations of thyme essential oil became moldy during the storage time due to the decrease in the number of starters and the creation of a competitive medium between the microorganisms.

4. Conclusion

In this research, active microcapsules produced with cress seed gum and Acacia senegal gum containing the effective composition of thymol were used to maintain the quality and increase the shelf life of yogurt during the storage period. The assessment results revealed that samples with higher thymol concentration in the microcapsules scored better in texture, taste, and overall acceptance at the end of the storage period. According to the results from the microbial counts, syneresis rate, sensory evaluations, and moldiness tests, yogurt samples containing microcapsules with cress seed gum (0.5%) and thymol (0.5%) had the longest storage duration and the best quality compared to other samples throughout the storage period. It is recommended to use 5% cress seed gum as a natural preservative in thymol-based active microcapsules to prolong the shelf life of yogurt when stored in the refrigerator.

Conflict of interest

The authors declare that there is no conflict of interest.

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