Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Microencapsulation of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* bacteria in sodium caseinate and pectin coating

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ARTICLE INFO

Article History:
Received:2024/4/22
Accepted:2024/7/3

Keywords:

Streptococcus thermophilus,
Lactobacillus bulgaricus,
coacervate,
microencapsulation,
sodium caseinate,
pectin

DOI: 10.22034/FSCT.21.156.137.

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ABSTRACT

The aim of the current research was microencapsulation of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* bacteria by coacervation complex method and sodium caseinate and pectin coatings. Sodium caseinate and pectin have electrostatic attraction together at pH=4 and form a complex. Using a scanning electron microscope, the morphology of coacervates was investigated. The properties of pectin and sodium caseinate complexes were investigated by infrared spectrometry tests and measuring the size and distribution of particles. Physicochemical characteristics such as water absorption, pH and acidity and sensory characteristics were also investigated. The results showed that the efficiency of microencapsulation of bacteria by coacervation method was 66.6%. The particle size was 1.565 micrometers and the zeta potential was reported as -16 mV. Electron microscope images showed spherical coacervate without holes and wrinkles on the surface. The results of infrared spectroscopy also showed the creation of electrostatic interactions between pectin and sodium caseinate. Also, microencapsulation of bacteria caused a significant change ($p>0.05$) in pH (4.6 ± 0.091) and yogurt acidity (1.1 ± 0.03) compared to pH (4.6 ± 0.1). And the acidity of yogurt (0.8±0.01) produced with free bacteria did not. The water content of yogurts produced with microencapsulated bacteria (40±1.1) was reduced compared to yogurts produced with free bacteria (45±0.9) ($p<0.05$). Bacterial encapsulation has a negative effect on color sensory characteristics. It did not have the smell and taste of the produced yogurts ($p>0/05$), but a significant difference was observed in the texture of the yogurts produced with microencapsulated bacteria compared to the yogurts produced with free bacteria ($p<0/05$).

[DOI: 10.22034/FSCT.21.156.137]

1- Introduction

Yogurt is a dairy product obtained from milk fermentation and has a special importance in the diet of people all over the world [1,2]. This dairy product is the result of the fermentation of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* [3].

The general characteristics of yogurt such as acidity, the amount of free fatty acid, amount of aromatic compounds (diacetyl, acetaldehyde and acetoin) along with sensory and nutritional characteristics are important features of this product. These characteristics are affected by the chemical composition of primary milk, process conditions, adding flavorings and the activity of starter bacteria during fermentation. Microencapsulation is a process in which a bioactive substance is covered by another substance (made of biopolymer, synthetic polymer or lipid compounds) and the formed small capsules can release their contents in a controlled manner under certain conditions. Protect the contents from destruction by harmful factors of the environment [4]. Microencapsulation increases survival, improves cell fermentation performance, increases production and recovery of metabolites (ethanol, lactic acid, riboflavin) [5,6]. Recently, microencapsulation of bacteria, especially probiotics, in foods, especially fermented foods, has received more attention. Prisco et al.[14] investigated the microencapsulation of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, as well as the performance and targeted release in the digestive tract, which showed the results of the survival of bacteria during storage and increased quality of yogurt. Also, the microencapsulation of Streptococcus *thermophilus* and *Lactobacillus delbruecki* was investigated by Janter et al. The investigation of microencapsulation of yogurt starter cultures by Hassan et al. [15] showed a higher viscosity and a higher stability index of yogurts with encapsulated starter bacteria. Choosing the right wall material and the right micro-encapsulation method is essential to achieve a successful microencapsulation. One of the microencapsulation methods that is very suitable for sensitive compounds is complex coacervation. The basis of this method is to create electrostatic attraction between two macromolecules with opposite charges [16]. Proteins and polysaccharides are widely used for wall materials in this method. Anionic polysaccharides have a negative charge due to having carboxyl, phosphate and sulfate groups, and proteins have a positive charge in the pH range lower than the isoelectric point. When this protein and polysaccharide are mixed together in a liquid environment, an electrostatic bond is created between the opposite charges and they neutralize each other, as a result, the bulk phase of the polymers and the supernatant phase are separated and sediment is formed [17, 18]. Various factors such as type of polysaccharide and protein, pH and ionic strength, molar ratio of two biopolymers and acidification stage are effective on the size of the particles and the properties of the complex formed [17].

Casein includes 75-80% of milk protein, which exists in the form of micelles in milk with various interactions, including electrostatic, hydrogen and hydrophobic interactions bind to polysaccharides. In industry, by adding organic and mineral acids to the milk, casein is precipitated and caseinate is produced after several stages, which increases the stability and solubility of caseins [19, 20].

Pectin is an anionic heteropolysaccharide found in plant cell walls and has a branched structure. Its main chain consists of galacturonic acid units with α-D (1:4) bond and is relatively esterified with methanol. In the branched part, it contains rhamnose, arabinose, galactan and galacturonic acid. Research shows that pectin is placed on the surface of casein with a positive charge in an acidic environment, and by creating electrostatic repulsion, it prevents casein from clumping, and causes particles to be stable in acidic environments [21, 22].

In addition to protecting bacteria from external factors, the microencapsulation of bacteria leads to higher cell density and is also effective in improving the performance of cell fermentation. When the bacteria are surrounded in the coating, the cell-cell contact becomes closer and the rate of gas release (i.e. oxygen) into the capsules also decreases, increasing the cell's tolerance to stressors. Also, the use of enclosed cells in fermentation processes such as yogurt increases the production of metabolites and improves the physicochemical properties of the product [10].

Therefore, the aim of this research is microencapsulation of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*

[DOI: 10.22034/FSCT.21.156.137]

bacteria (yogurt starter bacteria) by the complex coacervation method in sodium caseinate and pectin coating and also to investigate the effect of microencapsulation on physicochemical characteristics (pH, acidity and synersis) and sensory characteristics (color, taste and texture) is yogurt.

2- Material and Methods

1-2- Raw material

Sodium caseinate was purchased from Lactoprot (Riedlingen, Germany), pectin was purchased from CP KELCO (Brandenburg, Germany). Hydrochloric acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany), and De Man-Rogosa-Sharpe agar (MRS agar) culture medium was obtained from Iranian bio-exploration company. *Streptococcus thermophilus* (DSM 20617 1738) and *Lactobacillus bulgaricus* (DSM 20081 1737) bacteria were purchased from Iran Regional Center of Collection of Industrial Fungi and Bacteria (PTCC).

2-2- Preparation of bacterial culture

In order to prepare *Streptococcus thermophilus* and *Lactobacillus bulgaricus* bacteria, the vial of lyophilized bacterial powder was inoculated twice in MRS broth culture medium and kept in a greenhouse at 37°C for 24 to 48 hours. After reaching the desired number of cells, the resulting bacterial cells were centrifuged (Hermel, Germany) for 5 minutes at 4°C, and then the separated cells were washed twice with sterile phosphate buffer solution (pH = 8.6). and then it was used for microencapsulation.

3-2- Preparation of sodium caseinate and pectin complex

Sodium caseinate solution (1% w/v) and pectin (0.45% w/v) were mixed using a magnetic stirrer (Iran-PIT300) at 200 rpm until complete dissolution. After complete dissolution, the solutions were sterilized by autoclave (Radteb-Iran). Then the prepared solutions were kept at refrigerator temperature for 24 hours for maximum water absorption. In the next step, two solutions of sodium caseinate and pectin were mixed together and stirred for 10 minutes with a magnetic stirrer, and then bacteria were added to this solution and the pH of the solution was brought to 4 with 0.1 normal hydrochloric acid to precipitate [23].

4-2- Microencapsulation efficiency

1 gram of coacervate was added to 9 ml of phosphate buffer and stirred at high speed on a magnetic stirrer to destroy coacervates and release bacteria. Then the microencapsulation efficiency was calculated according to the following formula [24]:

(Relation 1)

= (released microcapsules of bacteria population - supernatant in unenclosed and free bacteria population) / (released microcapsules of bacteria population) efficiency of microcapsulation

5-2-Morphology

The surface morphology of the formed coacervates was investigated with a scanning electron microscope (Sigma, Germany).

6-2- Particle size, polydispersity index and zeta potential

To measure the average particle size, polydispersity index and zeta potential using dynamic light scattering (Antonpaar, Austria), coacervate were mixed with distilled water to form a suspension with a maximum concentration of 1% by weight, and then the samples were analyzed.

7-2- Fourier transform infrared spectroscopy

For spectroscopic analysis, the samples were mixed with potassium bromide and prepared in the form of tablets, and the spectrum of the samples was measured in the wavelength range of 400 to 4000 cm^{-1} (Cary 630, Germany).

8-2- yogurt production

Pasteurized and homogenized milk containing 3% fat was obtained from Pajan factory (Tehran-Iran) and heated at 90°C for 5 minutes and after cooling it was stored at 42°C. Then coacervates containing yogurt starter bacteria were added and kept in a incubator at a temperature of 42°C until a yogurt formed and the pH reached 4/6.

9-2-Acidity and pH

A pH meter was used to determine pH. To measure the acidity, 10 grams of the sample was diluted with 10 ml of water, then the acidity was determined through titration with 0.1 normal interest in the presence of the indicator

[DOI: 10.22034/FSCT.21.156.137]

phenolphthalein until the purple color appeared [24].

10-2- Syneresis

The amount of 20 grams of yogurt was weighed in a filter paper on a funnel, and after two hours in the refrigerator, the amount of water removed was weighed and the percentage of water was calculated according to the following formula [25].

(Relation 2)

(Drained weight-yogurt sample weight)/(Yogurt sample weight) x 100=watering percentage

11-2- Sensory evaluation

Sensory evaluation of yogurt samples was done by a panel of 6 people consisting of 4 women and 2 men in the age range of 25 to 35 years. Points were considered based on hedonic scoring from 1 to 10 (10 points for the best and 1 point for the worst).

12-2- Statistic analysis

Analysis of variance was used at a significance level of 5% to investigate the effect of microencapsulation of yogurt starter bacteria on the physicochemical and sensory characteristics of yogurt. The tests were performed in three repetitions and then the mean and standard deviation of the data were calculated. Data were analyzed with SPSS 20 statistical software.

3. Results and Discussion

1-3- Encapsulation efficiency

Encapsulation efficiency determines the ability of the microencapsulation method to trap core materials and is one of the important factors in determining the stability of encapsulated compounds. By increasing the efficiency of encapsulation, the stability and viability of compounds also increases [26]. pH and ionic strength are effective factors in the formation of complexes. Both affect the number of charges present on biopolymers and control the strength of electrostatic interactions [27]. Pectin is an anionic polysaccharide that has a negative charge. Sodium caseinate has both positive and negative charges due to having NH3+ and carboxyl CO- groups. Below the isoelectric point (pH=4.6), sodium caseinate has a positive charge. To form the complex, we brought the pH of the solution below the isoelectric point (pH=4) so that the positive charge of sodium caseinate and the negative charge of pectin have an electrostatic bond together and form a complex and enclose the bacteria in the complex [28]. The results of the present study showed the encapsulation efficiency of 66.6%. Da Silva et al.[27] reported the efficiency of *Lactobacillus acidophilus* with the coacervation complex method of 68.20-72.97%. Also, Chavari et al. reported the efficiency of microencapsulation of *Bifidiobacterium bifidi* microencapsulated by the coacervation method as 19.5-29.40% [29]. The efficiency of encapsulation is affected by the ratio of biopolymers and their charge density. If the charge ratio between pectin and sodium caseinate is suitable, the density of their different charges in the environment will be equal and pectin will completely cover the surface of sodium caseinate, and the force of attraction will be formed between the different charges and the formation of coacervates will take place [30, 31]. According to the results of microencapsulation efficiency of pectin in the ratio of 0.45% and sodium caseinate in the ratio of 1%.

2-3- Morphology

To know the morphology of the structure of coacervates, the surface morphology of the samples is shown by scanning electron microscope. In general, the produced coacervates had a compact and lumpy and spherical structure, and the surface of the samples was smooth and without holes and wrinkles. When the electrostatic attraction between two polymers is suitable, the number of coacervates formed is large and a mass structure is formed, and when the attraction is strong, the sealing of the capsules is complete and without any holes, which indicates the proper protection of the coatings from the target substance [32]. These results (Figure 1) were in line with the results of Saravanan et al. [33] who used pectin and gelatin as coating materials in the complex coacervation method. Of course, the presence of carbohydrates (pectin) is also effective in agglomeration. Similar results were reported by Peng et al. [29] and Sutank et al. [34, 35] when they used carbohydrates in the wall composition.

[DOI: 10.22034/FSCT.21.156.137]

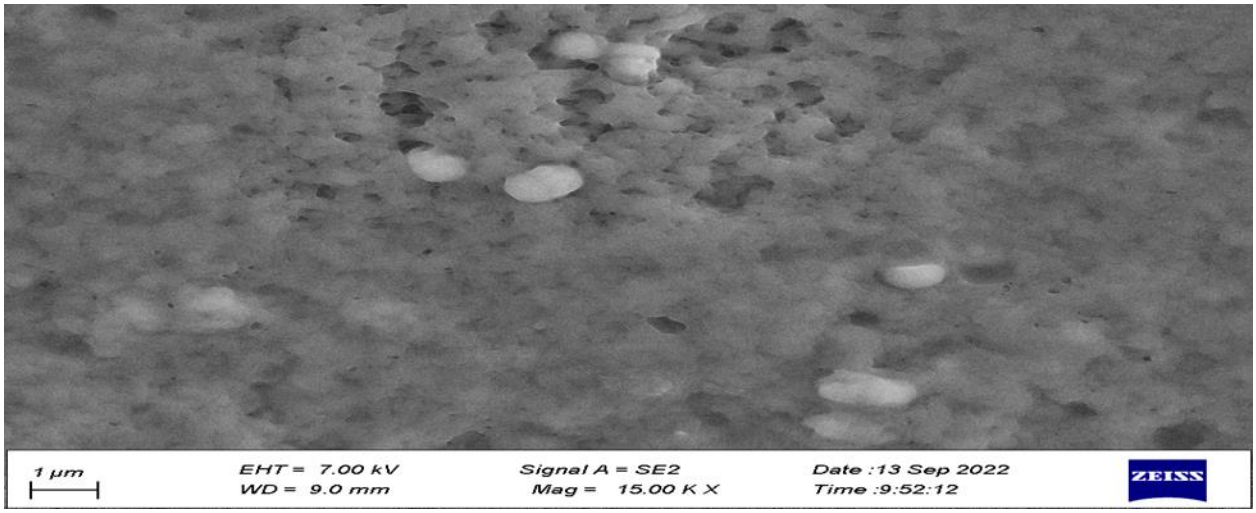

Figure 1- Surface morphology of pectin and sodium caseinate coacervate containing yogurt starter bacteria determined using scanning electron microscop

3-3- Measurement of particle size and zeta potential

In the coacervation method, the obtained particles have a size between 1 and 500 µm, and the particles that are smaller than 100 µm are suitable for use in food [36]. Particle size affects the sensory properties of the product. Particles that are large in size cause a harsh taste that is completely felt under the tongue, which is unpleasant for the consumer [37]. The size of the particles obtained for the ratio of sodium caseinate 1% and pectin 0.45% was 1.565 µm. Considering that the size of the particles in this study was smaller than 100 micrometers, it makes them interesting for use in food products.

Zeta potential is a function of pH and ionic strength and determines the stability of particle. When the goal is to form a coacervation complex, the zeta point should be close to zero (between +30 and -30 mV) because the strong electrostatic forces between the protein and polysaccharide charges promote complex formation. When the complex with maximum stability is formed, pectin completely covers the surface of sodium caseinate and most of the negative charge of pectin reacts with the positive charge of caseinate and forms a precipitate. As a result, the amount of free pectin is very low and the zeta potential is very low and close to zero. is [38]. The zeta potential obtained for the ratio of 1% sodium caseinate and 0.45% pectin was -16 mV, which confirmed the formation of a complex between sodium caseinate and pectin. Wang et al. (2019) reported the zeta potential of the sodium caseinate and pectin complex at pH = 4, which is below the isoelectric point and the complex is formed, as -5 mV [39].

4-3- Fourier transform infrared spectroscopy

Infrared spectroscopy is a practical and useful technique for studying the structure and interactions of protein-polysaccharide systems. When functional groups react at the molecular level, infrared spectroscopic changes such as the appearance of new bands and changes in the intensity and location of absorption bands are visible.

In the infrared spectroscopy of sodium caseinate, o-H and N-H stretch bonds showed a large peak in the wave number range of 3100cm^{-1} to 3300cm^{-1}. The observed peak at the wave number of 2956cm^{-1} indicates C–H stretching bonds. The wavenumbers were 1629cm^{-1},1511 cm^{-1}corresponding to amide I and amide II groups, respectively. The absorption peaks in the wave number of 1387 to 1442cm^{-1} were related to the C-H stretching vibrations of the amide III group [40, 41].

In the spectroscopic analysis of the sodium caseinate and pectin complex, the peak observed in the wave number range of 3328cm^{-1} is related to hydrogen bonds, and since it is the sum of the hydrogen bonds of sodium caseinate and pectin, as a result, the intensity of the bond also increased. The removal of the 2930cm^{-1} peak in sodium caseinate in the spectrum of the complex indicates the creation of an electrostatic bond between the amino groups of the protein and the carboxyl groups of pectin. The transfer of bonds of the amide I group of sodium caseinate from

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wave number 1629 and the carboxyl group of pectin from wave number 1604cm^{-1} to wave number 1623cm^{-1} in the complexes confirms the electrostatic interaction between sodium caseinate and pectin. In general, the shift of peaks and the emergence of new peaks in the spectrum of complexes indicate the confirmation of the interaction between sodium caseinate and pectin.

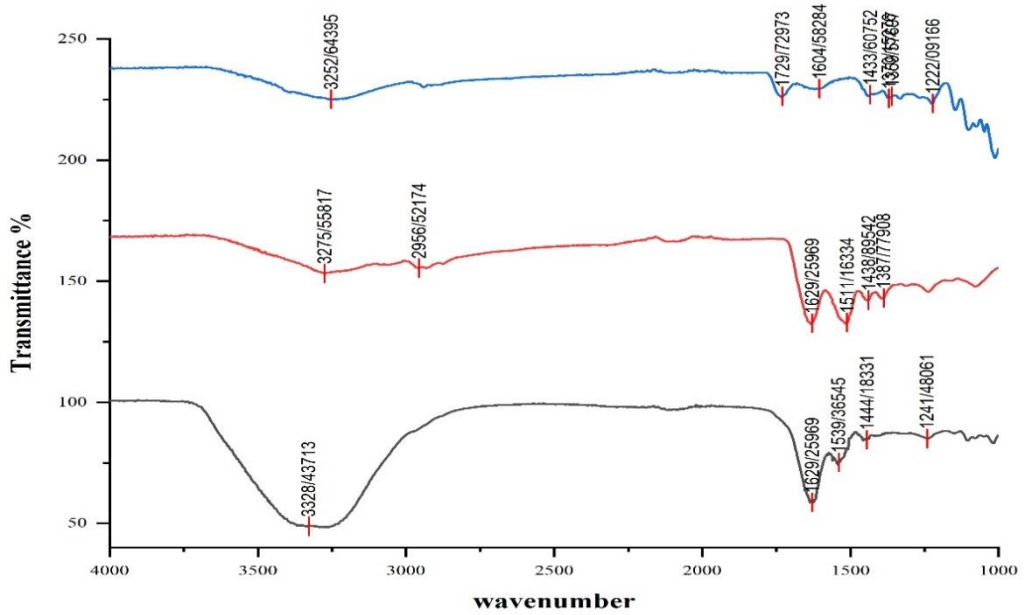

Figure 2-Ftir of pectin (a), sodium caseinate (b), and coacervate (c) containing yogurt starter bacteria

3-5- Physicochemical properties of yogurt produced with microencapsulated bacteria

1-3-5- Ph and acidity

Yogurt starter bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) live in symbiosis and there is a synergy between these two bacteria that is related to a mutual stimulation. This stimulation leads to growth, acidification and production of aromatic compounds. Both species are fermenting bacteria that produce lactic acid from milk lactose. The production of lactic acid leads to a decrease in pH. According to the obtained results, encapsulation of yogurt starter bacteria with sodium caseinate and pectin coating did not have a negative effect on the pH of the produced yogurt sample and there was a significant difference (P>0.05). It was not observed in the pH of yogurt samples produced with micro-coated and free bacteria. These results showed that the microencapsulation of yogurt starter bacteria did not have a negative effect on the quality of yogurt samples and the pH of the treatment was in line with the pH determined by the National Standard Organization of Iran (maximum level 4.6) (Iranian National Standard No. 695, 1401). Microencapsulation of yogurt starter bacteria increased the acidity of yogurt samples produced with microencapsulated bacteria compared to samples containing free bacteria, but it was not statistically significant (P>0.05). The increase of whole milk solid matter increases the growth of yogurt starter bacteria and as a result the final acidity of the product increases slightly. The results of this study are consistent with the results of Spiritosanto et al. [33].

Table 1- The pH, acidity and percentage of yogurt produced with microencapsulated and non-encapsulated bacteria

Physicochemical properties	A sample of yogurt produced with microencapsulated bacteria by sodium caseinate and pectin	A sample of yogurt produced with unencapsulated starter bacteria
pH	4/6±0/091	4/6±0/1

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Acidity	1/1±0/03	0/8±0/01
Syneresis (%)	40±1/1	45±0/9

The numbers are the average of three repetitions and are the mean ± standard deviation.

Significant difference at probability level 5%*

2-3-5- Syneresis

Syneresis is the removal of liquid from the yogurt gel matrix due to the shrinkage of the protein structure, which leads to a decrease in the strength of whey proteins and the separation of serum, which affects the quality of yogurt. syneresis is one of the factors that affect the quality of the product. In this research, the use of sodium caseinate and pectin coating reduced the water content of yogurt compared to the sample without sodium caseinate and pectin and it reached 401±1.1% ($P < 0.05$). In the presence of sodium caseinate and pectin, the liquefaction of yogurt decreases, which is due to the increase of its dry matter. The increase of dry matter increases the consistency, stiffness and elasticity of the coagulate and reduces the amount of water loss. According to the results of Aziznia et al.[31], with the increase of dry matter, the water content of yogurt decreased. Pectin is an anionic hydrocolloid that has gelling properties and excellent water retention. In fact, pectin forms a three-dimensional network that is able to interact with positive charges on the surface of proteins and has maximum water absorption, which reduces synergism [32]. The reduction of yogurt liquefaction in the presence of pectin was also reported by Jensen et al.[21]. Sodium caseinate improves the stability (increase in viscosity) and reduces water loss by strengthening the structure of the protein network in yogurt. Rezaei et al. [43] reported that adding sodium caseinate to yogurt can be effective in reducing water retention during storage.

6-3- Sensory evaluation

The sensory characteristics of yogurt are the most important reason for the popularity of this product among consumers. According to the national standard of Iran, sensory factors that are important in yogurt include color, smell, taste, and texture. Sensory evaluation of yogurt produced with microencapsulated bacteria compared to yogurt produced with non-encapsulated bacteria is shown in Table 3. According to the results, the color of yogurt produced with microencapsulated bacteria had a score of 9.86 ± 0.51, which was not significantly different from the color of yogurt produced with free bacteria ($P < 0.05$). The odor score of yogurt produced with encapsulated bacteria was reported as 9.15 ± 0.98 ($P < 0.05$). Also, no significant difference was observed in the taste of yogurt produced with microencapsulated bacteria ($P < 0.05$). The results showed that sodium caseinate and pectin had no negative effect on the color, smell and taste of yogurt. But there was a significant difference in the texture of yogurt produced with microencapsulated bacteria and yogurt produced with free bacteria ($P < 0.05$) and the score of yogurt produced with microencapsulated bacteria was 6.10±0.89 and for yogurt produced with Free bacteria was reported as 7.73 ± 0.4, because yogurt produced with microencapsulated bacteria had less syneresis and better texture due to more dry matter (sodium caseinate and pectin) because pectin has gelling and thickening properties and even in low concentrations, it absorbs water and increases viscosity and hardness. Also, due to the thermal process applied to the milk (90°C, 5 minutes), a gel-like structure with a cross microstructure is formed by denatured whey proteins associated with casein micelles, which controls the gel strength and yogurt texture. Therefore, higher protein content is effective in creating a stronger gel network and makes the product firmer, thus improving the yogurt texture.

Table 2- sensorial evaluation of yogurt produced with microencapsulated and non-encapsulated bacteria

Physicochemical properties	A sample of yogurt produced with microencapsulated bacteria by sodium caseinate and pectin	A sample of yogurt produced with unencapsulated starter bacteria

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Colour 1-10	9/86±0/51	9/60±0/54
Flavor 1-10	9/15±0/98	9/00±0/89
Texture 1-10	6/10±0/89	7/73±0/4
General acceptance 1-10	6/00±0/83	7/5±0/5

The numbers are the average of three repetitions and are the mean ± standard deviation.

Significant difference at probability level 5%*

4-Conclusions

This research was carried out with the aim of microencapsulating yogurt starter bacteria using complex coacervation technique with sodium caseinate and pectin. The results showed that pectin at a concentration of 0.45% and sodium caseinate at a concentration of 1% can form dense complexes with spherical morphology that can protect the target substance well. The size of coacervates was reported to be 1.565 micrometers, which showed that they are suitable for use in food. Infrared spectroscopic analysis confirmed the electrostatic interaction between sodium caseinate and pectin. Also, the results showed that the microencapsulation of starter bacteria did not have a negative effect on the physicochemical and sensory characteristics of the produced yogurt. As a group of natural biopolymers, sodium caseinate has excellent digestibility and low toxicity, and pectin has therapeutic benefits such as reducing blood cholesterol, removing heavy metal ions from the body, stabilizing blood pressure and facilitating intestinal activity, and in the pharmaceutical industry. It has many uses. As a result, the coatings used do not have negative effects on health, but they are also beneficial for health and can be used to cover yogurt starter bacteria and improve the quality of yogurt. Of course, this claim requires additional studies, including the interaction between bacteria and coating compounds. Also, in order to improve the quality and health of yogurt, it is recommended to use the microencapsulation of other probiotic bacteria, and the design of controlled release systems of probiotics during the maintenance and enhancement of the health-giving effect of yogurt is also recommended.

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مجله علوم و صنایع غذایی ایران

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مقاله علمی_پژوهشی

تاثیر ریزپوشانی باکتری‌های *استرپتوکوکوس ترموفیلوس* و *لاکتوباسیلوس بولگاریکوس* بر خصوصیات فیزیکوشیمیایی و حسی ماست

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۳/۲/۳

تاریخ پذیرش: ۱۴۰۳/٤/۱۳

کلمات کلیدی:

استرپتوکوکوس ترموفیلوس،

لاکتوباسیلوس بولگاریکوس،

کواسروات،

ریزپوشانی،

کازئینات سدیم،

پکتین

DOI:10.22034/FSCT.21.156.137.

* مسئول مکاتبات:

چکیده

هدف از پژوهش حاضر ریزپوشانی باکتری‌های *استرپتوکوکوس ترموفیلوس* و *لاکتوباسیلوس بولگاریکوس* با روش کمپلکس کواسرواسیون و پوشش‌های کازئینات سدیم و پکتین بود. کازئینات سدیم و پکتین در pH=٤ با هم جاذبه الکترواستاتیکی داشته و کمپلکس تشکیل می‌دهند. با استفاده از میکروسکوپ الکترونی روبشی، مورفولوژی کواسروات‌ها بررسی شد. ویژگی‌های کمپلکس‌های پکتین و کازئینات سدیم توسط آزمون‌های طیف‌سنجی فروسرخ و اندازه‌گیری اندازه و توزیع ذرات بررسی شد. ویژگی‌های فیزیکوشیمیایی همچون آب‌اندازی، pH و اسیدیته و ویژگی حسی نیز بررسی گردید. نتایج نشان داد، راندمان ریزپوشانی باکتری‌ها با روش کواسرواسیون ٦٦/٦درصد بود. اندازه ذرات ۱/۵٦۵ میکرومتر و پتانسیل زتا ۱٦- میلی ولت گزارش شد. تصاویر میکروسکوپ الکترونی، کواسروات‌هایی کروی شکل و بدون سوراخ و چروک در سطح را نشان داد. نتایج طیف‌سنجی مادون قرمز نیز ایجاد برهمکنش‌های الکتروستاتیک بین پکتین و کازئینات‌سدیم را نشان داد. همچنین ریزپوشانی باکتری‌ها موجب تغییر معنی داری ($p>0/05$) در pH(0/091 ± ٤/٦)و اسیدیته ماست (۱/۰۳±۱/۰) در مقایسه با pH (۰/۱ ±٤/٦)و اسیدیته ماست (۰/۰۱±۰/۸)تولید شده با باکتری های آزاد، نشد. آب‌اندازی ماست‌های تولید شده با باکتری‌های ریزپوشانی (40±1/1درصد) در مقایسه با ماست های تولید شده با باکتری‌های آزاد (۰/۹ ± ٤۵درصد) کاهش یافت ($p<0/05$). ریزپوشانی باکتری‌ها تاثیر منفی بر ویژگی‌های حسی همچون رنگ، بو و مزه ماست‌های تولید شده نداشت($p>0/05$) ولی تفاوت معنی‌داری در بافت ماست‌های تولید شده با باکتری‌های ریزپوشانی شده در مقایسه با ماست‌های تولید شده با باکتری‌های آزاد مشاهده شد ($p<0/05$).

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