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Fortification of yogurt with encapsulated chia seed oil and mucilage: optimization and evaluation of physicochemical and rheological properties

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ABSTRACT

The aim of the present study was to optimization of enriched yogurt with chia seed oil and mucilage. This purpose was done with the aim of maximizing omega-3 level and minimizing changes in physical and chemical properties (pH, titrate acidity, syneresis, dry matter, peroxide values and color changes) compared to the control sample. First, in order to increase the oxidative stability, chia seed oil was encapsulated with sodium alginate and chia seed mucilage using emulsification/ internal gelation method. The physical characteristics results showed that the enrichment caused a decrease in the syneresis, an increase in the water holding capacity and viscosity. Then, yogurt was enriched and optimized with free and encapsulated chia seed oil (0 to 4%) and chia seed mucilage (0 to 0.25%). The sample without chia seed mucilage and oil used as a control sample. The results of scanning electron microscopy images showed that the successful alginate-sodium-mucilage microfiber coating of chia seeds has been made with a uniform structure. Yogurt contained 3.41% encapsulated chia seed oil and 0.213% chia seed mucilage was selected as an optimum treatment. The results showed that the number of starter bacteria of optimum sample were not significantly different from the control ($p>0.05$). The texture of the yogurts was found to be viscoelastic. The flow behavior index in all samples was less than one, which indicates the non-Newtonian (pseudoplastic) behavior of the samples. Herschel Balky model had R^2 and lowest RMSE in both samples, which indicates the suitability of this model to investigate the shear flow behavior in terms of shear velocity of samples.

1. Introduction

Chia is from the Spanish word Chiyin[1] or what[2] It is adapted from Nahuatl[3] It means oily [1]. Chia seeds from the plant*Salvia hispanica*[4] From the Lamiaceae family[5] and sage are obtained [2]. These seeds contain 18-24% protein, 25-50% oil, about 42% carbohydrate and about 5% ash. Chia seeds are a rich source of compounds with high biological activity, especially polyphenols such as gallic acid.[6]Caffeic acid[7]Chlorogenic acid[8], cinnamic acid[9]Frolic[10]Querstein[11], Comperful[12]Ipacatechin[13], routine[14]Apigenin[15] and picumaric acid[16] are also These seeds also contain plant sterols such as campesterol, stigmasterol, cystosterol, tocopherols, minerals and vitamins [2-5]. Chia seed oil contains large amounts of unsaturated fatty acids, especially alpha-linolenic acid from the group of omega-3 fatty acids, which plays an important role in preventing cardiovascular diseases, especially in people at risk. [6-10]. But one of the problems of polyunsaturated fatty acids is their lack of oxidative stability, which can be used to protect them by methods such as encapsulation.

Encapsulation is considered as a useful tool to improve the storage and delivery of bioactive compounds and provides the possibility to release them at a specific time, place or product while maintaining these compounds [11, 12]. In recent years, various methods have been developed for the encapsulation of bioactive compounds.Emulsification method is widely used due to its ease, speed, safety for food uses and availability. In this method, usually drops of oil or water throughDispersing one liquid in another immiscible liquid is produced [13-15]. One of the upcoming challenges in the field of interior cladding is choosing suitable wall materials with maximum stability against external factors over time. Polysaccharides are mainly used as wall materials. Sodium alginate[17] It is a linear polysaccharide derived from alginic acid due to its thickening ability[18], the property of film formation[19], sustainability, biocompatibility, cheapness and availability are used in the development of microcoating technology. Sodium alginate is easily dissolved in water, but it forms a gel when placed in the vicinity of calcium ions, which is due to the interaction with divalent cations as a cross-linking agent, which causes the linking of functional groups and the formation of a gel network, which is a method for the production of microcoatings. is [11, 16, 17].

Chia seeds contain approximately 30-34% dietary fiber. Fiber is a complex composition including oligosaccharides and polysaccharides such as cellulose and hemicellulose, which have high water absorption properties and the ability to form gels due to the presence of carbohydrates with free polar groups. In the food industry, fibers as a foam stabilizer[20], suspension factor[21], emulsifier and texture improver are used. Lignin is the main insoluble part of fiber that plays an important role in protecting unsaturated fatty acids. The soluble part of the fiber partially leaves the seed and forms a gel in contact with water. Part of the fiber in chia, which is located in the outer part, after absorbing water, is partially removed from the surface of the seed and appears in the form of mucilage, which completely adheres to the seed itself [3, 18]. One of the ways to improve the nutritional value of food is enrichment with bioactive compounds [19].. Of the food Different, Due to the high consumption in the world, dairy products are considered more as a food base for enrichment [20]. Due to the expansion of the production and consumption of all kinds of yogurt and on the other hand not having enough bioactive compounds [21], enriching this product with useful compounds including essential fatty acids can be very useful. Since no research has been done on the use of chia seed oil in free and microencapsulated form in yogurt and its effect on its qualitative,

1. . . . Chian
2. Dog
3. Nahuati
4. *Sage*
5. Lamiaceae
6. Gallic acid
7. Caffeic acid
8. Chlorogenic acid
9. Cinnamic acid
10. Ferulic acids
11. Quercetin
12. Kaempferol
13. Epicatechin
14. Routine
15. Apigenin
16. P-coumaric acid
17. Sodium alginate
18. Thickening
19. Film-formingproperty
20. Foam stabiliser
21. Suspending agent

nutritional and rheological characteristics, the present research aims to microencapsulate chia seed oil using sodium alginate. and chia seed mucilage with the help of internal emulsification-gelation method in order to protect it oxidatively and then optimallyMaking yogurt enriched withMicro-encapsulated chia seed oil, free oil and chia seed mucilage based on physical and chemical characteristics (pH, acidity, peroxide, dry matter, hydration, color and omega 3) and finally the comparison of the optimal sample with the control in terms of the number of initiator bacteria and rheological characteristics was done. .

2- Materials and methods

2-1- Materials

Pasteurized milk and high-fat yogurt (Mihen, Iran) and chia seeds were obtained from Zanjan local market. All chemicals with laboratory grade purity and culture media were obtained from Merck (Germany). Sodium alginate was obtained from Sigma Aldridge (USA).

2-2- Chia seed oil and mucilage extraction

To extract mucilage, 100 g of chia seeds were mixed with 2000 ml of distilled water (1:20 (w/v)) and stirred using a magnetic stirrer (100 rpm) for 45 min at 50 ± 2 °C for 45 It was stirred for a minute. After cooling the final mixture and storing it overnight in the refrigerator (4 degrees Celsius) in order to fully dehydrate the seeds, it was poured into metal trays and placed in an oven at 50 degrees Celsius for 24 hours to dry. After storing the seeds for 24 hours at ambient temperature to create moisture balance, the dried mucilage layer was separated by a sieve with 25 mesh. The resulting powder was sealed in glass containers impermeable to moisture and air and kept at room temperature until use [18, 22]. Chia seed oil was extracted using a cold hydraulic press (Nord company, model KK15, Germany) at a pressure and temperature of 20 MPa and 25 degrees Celsius, respectively. To prevent oxidation, the resulting oil was poured into moisture and air-proof containers and kept at a temperature of 4 degrees Celsius [23].

2-3- Microcoating of chia seed oil and evaluation of microcoatings

First, 2 grams of equal mixture of chia mucilage powder and sodium alginate in 100 ml of distilled water (ratio 1:50 (weight/volume))It was dissolved and after stirring for 2 hours at room temperature, the mixture was kept overnight in the refrigerator (4 degrees Celsius). Micro-coating process using internal emulsification-gelation method [13] through complete mixing30 g of this mixture was mixed with 10 g of chia seed oil (6 min at 3000 rpm). in order to formMicrocoatings The resulting emulsion was added drop by drop to the calcium chloride solution (1% (weight/volume)) and The resulting microcoatings were washed with 0.05 M calcium chloride solution and separated by a nitrocellulose filter with the help of Millipore glass vacuum system.It was poured into distilled water at a ratio of 1 to 9 and kept at 4 degrees Celsius until use. and kept at 4 degrees Celsius until use. To measure emulsion stability (IS)[22], 100 ml of emulsion (IN_0) was transferred to a graduated cylinder and kept for one hour at room temperature [13, 15]:

$$ES\ (\%) = (V/V_0) \times 100 \quad (1)$$

which equals VThe oily phase is separated after one hour.Calculating microcoating efficiency[23] (EE) using the initial mass of the used oil (M_0) and the mass of non-microcoated oil (M) remaining on the surface as well as on the surface of the microcoatings [13, 22]:

$$ES\ (\%) = (M_0 - M / M_0) \times 100 \quad (2)$$

In order to ensure the formation of microcoatings and to examine their morphology with a scanning electron microscope[24] was used In this way, after drying the microcoatings with a freeze dryer, the resulting powder was fixed on the surface of the scanning electron microscope, and after coating with gold (6-11 nm, 10 mA and 40 seconds) into the electron microscope device, which voltage Acceleration of electrons was 15 kV and the image was taken [17, 18].

2-4- Yogurt preparation

3% of yogurt was added to pasteurized milk at a temperature of 45 degrees Celsius, and after adding chia seed oil (0-4%) in a free or micro-coated form andMucilage (0-0.25%) was mixed with it at low speed depending on the treatment (Table 1). The control sample was considered as the sample without chia seed oil and mucilage. In the following, fermented milk untilReaching pH = 4.5 (about 3 hours) was placed in the oven with a temperature of 45 degrees Celsius and finally 24 hours inThe temperature is 4 degrees Celsius

22. Emulsion stability (%ES)

23. encapsulation efficiency(EE%)

24. Scanning electron microscope (SEM)

was kept

Table 1 Design matrix of the response surface methodology.

Run	Encapsulation	Chia seed musilage (%)	Chia seed oil (%)	Run	Encapsulation	Chia seed musilage (%)	Chia seed oil (%)
1	With	0.125	2	13	Without	0.037	0.59
2	Without	0.125	2	14	With	0.125	4
3	With	0.21	0.59	15	Without	0.125	2
4	With	0.21	3.41	16	Without	0.125	2
5	With	0.037	3.41	17	Without	0.125	2
6	With	0.037	0.59	18	With	0.125	2
7	With	0.125	2	19	Without	0.125	4
8	Without	0.21	3.41	20	Without	0.125	0
9	Without	0.25	2	21	With	0	2
10	With	0.125	2	22	Without	0	2
11	With	0.25	2	23	With	0.125	0
12	Without	0.037	3.41	24	Without	0.21	0.59
12	Without	0.037	3.41	24	Without	0.21	0.59

6-2-Optimization

To determine the optimal conditions, the numerical optimization method was used with the aim of maximizing the amount of omega-3 and simultaneously minimizing the changes in pH, acidity, dry matter, color characteristics, amount of watering and peroxide compared to the control sample.

Yogurt acidity and pH were measured by Tamjidi et al.'s method [24].

To measure the dry matter, about 3-5 grams of yogurt was weighed and placed in the oven at a temperature of 105 ± 2 degrees Celsius until a constant weight was reached, and the amount of dry matter was calculated from Equation 3 [8]:

Amount of dry matter (percentage)=M / M_0) × 100 (3)

where inM_0: The initial mass of our sample andM: The mass of the sample is dried.

To measure the amount of yogurt hydration, 30 grams of yogurt was placed on filter paper for 1 hour at 10 degrees Celsius and the percentage of yogurt hydration was calculated according to the weight of the water removed from it according to equation 4 [24]:

Irrigation amount (percentage) = (M / M_0) × 100 (4)

where inM_0: The initial mass of our sample andM: The weight of the water removed from the yogurt.

To measure the peroxide number of yogurt samples, first, 5 grams of yogurt and 30 ml of glacial acetic acid-chloroform solution (3:2 (volume/volume)) were mixed completely and 0.5 ml of saturated potassium iodide was added to it. After 10 minutes. Keeping the mixture in a dark environment, 30 ml of distilled water and 1 ml of starch glue (1%) were added to it and titrated with 0.01 M sodium thiosulfate, and the peroxide number was obtained from equation 5 [24]:

= Peroxide number (meq/kg) = [N(S-B) / M_0] × 100 (5)

where inM_0: the initial mass of our sample,N: Normality of sodium thiosulfate,:S The volume of sodium thiosulfate used for the sample andB: The volume of sodium thiosulfate used is for the witness. In order to determine the color indices, surface imaging was used in the image processing chamber with the help of a Canon EOS (70D) camera, and after extracting the color indices*(a^*□ b^*□ (L^**Using Image J software (1.40g, United States of America), the amount of color difference of the samples compared to the control sample (ΔE) was obtained from equation 6 [25]:

ΔE=

$$\sqrt{(L^* - L^*{}_0)^2 + (a^* - a^*{}_0)^2 + (b^* - b^*{}_0)^2}$$

$$\sqrt{(L^* - L^*{}_0)^2 + (a^* - a^*{}_0)^2 + (b^* - b^*{}_0)^2} \quad (6)$$

2-7- Comparing the optimal sample with the control

The comparison of optimal and control samples was done by checking the viability of starter bacteria and rheological evaluation of yogurt samples.In order to count the initiator bacteria, 0.1 ml of the concentration^{7-}10 yogurt samples to plates containing MRS culture media

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(*lactobacilli)* and M17 agar(*streptococci)* was moved. The plates at a temperature of 37 degrees Celsius respectively in anaerobic conditions for 72 hours andAerobic conditions were kept in a greenhouse for 48 hours and then counted [26].

Rheological evaluation of yogurt samples by dynamic shear rheometer (Anton paar, model MCRS1, Austria) with cone-plate geometry, 50 cm in diameter, 0.01 mm pitch/gap distance and 1 degree cone angle was used for rheological evaluation. Each sample was placed in the space between the cone and plate for 5 minutes to rest and recover the structure. The tests were performed at a temperature of 25 degrees Celsius and with three repetitions became. In order to determine the viscoelastic properties, first, a scan-strain oscillation test to determine the linear viscoelastic region.[25] and then the appropriate strain in the linear viscoelastic range (one percent) to perform the sweep-frequency test[26] Selected. The frequency scan test was performed in the frequency range of 0.01 to 100 Hz by applying a constant strain of 1%, and finally the loss and storage moduli were recorded by the device. To describe flow behavior from power law models[27] (Ref. 7), Bingham[28] (Relation 8), Herschel-Bulkelley[29] (Relation 9), Casson[30] (Relation 10) and Vocadlo[31] (Relation 11) was used:

$$\tau = K\dot{\gamma}^{n}\tau = K\dot{\gamma}^{n} \quad (7)$$

$$\tau = \mu_p\dot{\gamma} + \tau_0\tau = \mu_p\dot{\gamma} + \tau_0 \quad (8)$$

$$\tau = K\dot{\gamma}^{n} + \tau_0\tau = K\dot{\gamma}^{n} + \tau_0 \quad (9)$$

$$\sqrt{\tau} = \sqrt{\tau_0} + K_c\sqrt{\dot{\gamma}}\sqrt{\tau} = \sqrt{\tau_0} + K_c\sqrt{\dot{\gamma}} \quad (10)$$

$$\tau = [\tau_0{}^{1/n} + K_V\dot{\gamma}]^n\tau = [\tau_0{}^{1/n} + K_V\dot{\gamma}]^n \quad (11)$$

where τ equals the shear stress (Pa),$\dot{\gamma}\dot{\gamma}$ Equal to shear speed (s^{-1}), n is equal to flow behavior index, K is equal to consistency factor ($Pa.s^n$)، $_p\mu$ is Bingham's plastic viscosity (Pa.s),$_0\tau$ is equal to the yield stress (Pa), K_c and K_{IN} It is equal to the Casson model constant and the consistency index for the Vocadello model, respectively.

8-2-Statistical analysis

Optimization by Response Surface Method (RSM)[32] and with a central composite design (CCD)[33] With the help of two numerical factors including the concentration of chia seed oil (0 to 4 percent) and the concentration of mucilage (0 to 0.25 percent) and a qualitative factor, micro-covering was done. The upper and lower levels were chosen based on preliminary studies (for mucilage concentration) and taking into account the required amount provided by the omega-3 diet, the omega-3 content of chia seed oil, and the loss rate of chia seed oil. To compare the optimal sample and the control, a factorial design was used with a randomized complete block basic experimental design with at least three repetitions, and time was considered as a block.Evaluation and comparison of rheological models with the help of coefficient of explanation (R^2) and root mean square error (RMSE) were performed.

$$R^2 = 1 - \left(\frac{\sum_{i=1}^{n}(O-P)^2}{\sum_{i=1}^{n}(O-\bar{P})^2}\right)R^2 = 1 - \left(\frac{\sum_{i=1}^{n}(O-P)^2}{\sum_{i=1}^{n}(O-\bar{P})^2}\right) \quad (12)$$

$$\text{RMSE} = \sqrt{\frac{(O-P)^2}{n-p}}\text{RMSE} = \sqrt{\frac{(O-P)^2}{n-p}} \quad (13)$$

where O, P, p and n are experimental data, predicted data, number of observations and number of model variables, respectively.Analysis of optimization data was done with Design Expert software (version 12, USA) and comparison data was done using one-way ANOVA with the help of SPSS software (version 26, USA). Duncan's post hoc test was used to compare the means.

3. Results and Discussion

3-1-Evaluation of microcoating of sodium alginate-chia seed mucilage

The formation and stability of the emulsion is one of the most important steps in the microcoating process using the emulsification method, which

25. Linear viscoelastic region
26. Frequency sweep
27. Power Law
28. Bingham
29. Herschel-Bulckly
30.. Casson
31. Vocadlo
32. Response surface methodology (RSM)
33. Central composite design(CCD)

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has a significant effect on the efficiency of the microcoating as well as the quality of the final microcoating [13, 15]. The results of the emulsion stability investigation showed that the emulsion stability index was 98.6±0.1%, which indicates the complete stability of the emulsion. Adding sodium alginate as a hydrophilic hydrocolloid, on the one hand, increases the viscosity of the continuous phase around the oil droplets and limits their movement, and on the other hand, due to the absorption between the oil and water phase, it reduces the surface tension and emulsion stability [27]. The results of the present research showed that the efficiency of the emulsification method for the microcoating of chia seed oil is 89.8 ± 0.24%. Chan et al also reported microcoating efficiency between 85 and 90% for coating*Lactobacillus* obtained with sodium alginate coatings. They found that the efficiency of microcoating is dependent on the speed of formation of the coating wall due to cross connections in addition to alginate concentration and its type. Part of the free oil may be related to oil leakage from the surface of the microcoating prior to the formation of a continuous wall by the alginate phase [27]. Similar results have been reported by other researchers for alginate-based coatings [11, 13, 15, 17]. The results of the scanning electron microscope images show the successful formation of the alginate-mucilage microcoating of chia seeds, which is completely round and about 10 micrometers in size with a uniform shell structure (Figure 1). Also, the surface of the microcoating wall has pores that cause the slow release of the materials inside the core. Similar results have been reported by other researchers for alginate coatings [11-13, 15, 17, 27].

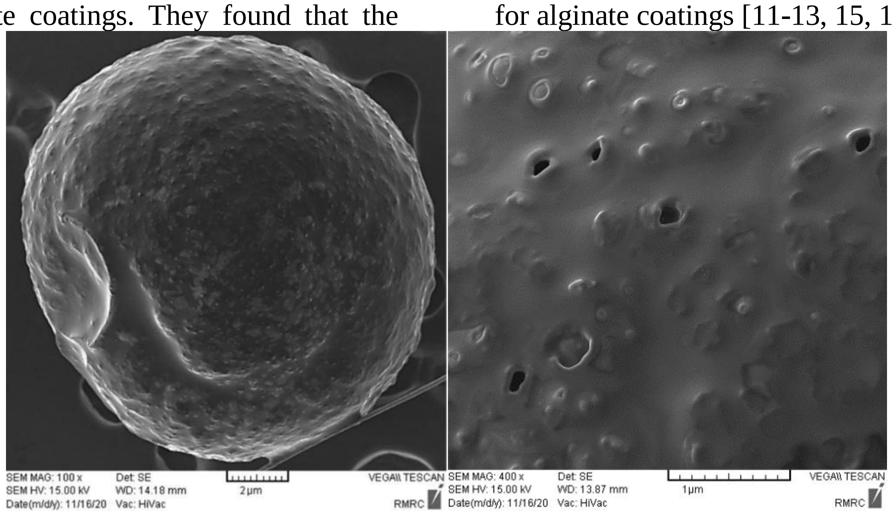

Fig 1 Scanning electron microscopy images from sodium alginate-chia seed mucilage containing chia seed oil

3-2-pH and acidity

pH and acidity affect the textural characteristics of yogurt gel and its storage time, and it is influenced by factors such as the chemical composition of milk, process conditions, additives, and the activity of initiator bacteria during fermentation [28]. According to the results, the pH of enriched yogurt samples varied between 4.31-4.66. The optimal pH for achieving the desired properties of yogurt texture has been reported as 1.4-4.6 [29]. Analyzing the results of variance analysis showed that the independent numerical variable of chia seed oil has a significant effect on the pH and acidity changes ($p<0.05$), so that according to Figure 2, with the increase in the amount of oil, the acidity of the samples decreased and their pH increased. Meanwhile, the addition of chia seed mucilage and micro-coated oil and the micro-coating process have no significant effect on their changes ($p>0.05$).

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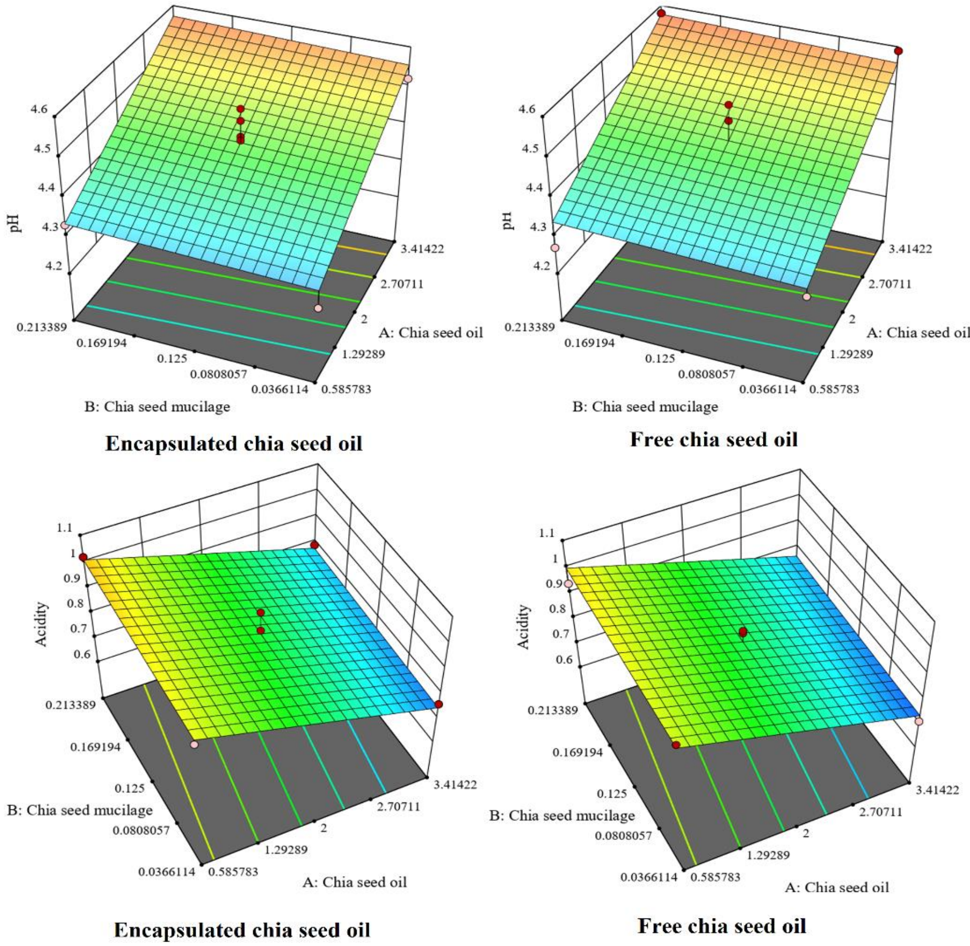

Fig 2 The change in pH and acidity of yogurt vs. independent variables.

In order to prove the effect of independent process variables on pH and acidity changes, from the perturbation or saturation diagram[34] was used (Figure 3). In the perturbation diagram, the partial changes of each variable around the central point are shown. A steep slope or curvature of a variable indicates that the response is sensitive to this variable, and a relatively flat line indicates the insensitivity of the response to changes in that particular factor [32, 33]. Among the studied numerical variables, since the slope of the graph of chia seed oil is higher than the slope of the graph of chia seed mucilage, it can be concluded that pH and acidity changes are more sensitive to chia seed oil than mucilage. The results of studies conducted in the past also showed a significant effect ($p<0.05$) of adding purslane oil [30], chia seed extract [31], chia seed and strawberry [8] on changes in pH and acidity of yogurt and no significant effect (0.05). p>) addition of different ratios of konjak and marvo gum have shown changes in yogurt pH and acidity [29].

[34]. Perturbation

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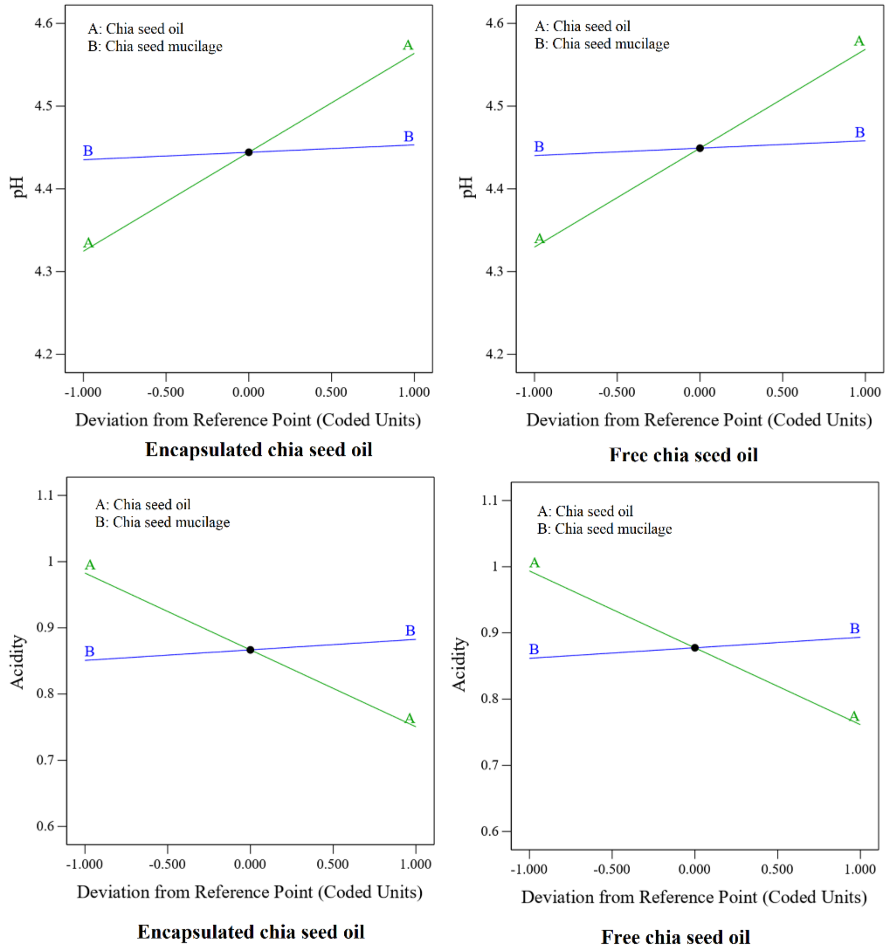

Fig 3 The perturbation plot of the changes in pH and acidity of yogurt.

3-3- Dry matter and watering

One of the most important goals of the dairy industry is to produce desirable yogurt with minimal water loss during transportation and storage. The structure of yogurt can be defined as a three-dimensional network of chains and clusters of casein micelles that have maintained their spherical shape. Watering generally occurs due to the change and failure of the protein network of yogurt, the wrinkling of its structure, and the reduction of the binding power of serum proteins to the casein network during storage and applying tension. The percentage of fat, the characteristics of starter bacteria, the amount of dry matter without fat, the addition of water absorbent materials such as fibers and its products, fermentation temperature and pH are among the most important factors to control the yogurt's wateriness [8, 26, 30]. The dry matter of yogurt is also effective in the consistency and amount of hydration of the final product.

The results of analysis of variance showed that the addition of chia seed oil has a significant effect on increasing the dry matter of yogurt samples ($p<0.05$). This expected increase, according to Figure 4, is due to the addition of oil to the yogurt formulation, which has changed the total dry matter in the range between 12.5 and 17.6 percent. However, oil coating and addition of mucilage has no significant effect on the amount of dry matter ($p>0.05$). Although total dry matter was expected to increase with increasing mucilage; But this can be attributed to the relatively low amount of added mucilage powder (between 0 and 0.25%) compared to chia seed oil and the large changes in dry matter (12.5 to 17.6%) under the influence of chia seed oil addition. which causes the changes caused by the addition of mucilage powder to not be significant ($p> 0.05$). However, as it is clear from Figure 4, the quadratic effect of this factor on the total dry matter of yogurt is significant ($p<0.05$). Also, the results of Figure 4 show that the addition of chia seed oil and its mucilage and the microcoating process have a significant decreasing effect on the water content of enriched yogurt samples ($p<0.05$); So that the amount of watering of the optimal sample decreased by 48.79% compared to the control sample. However, the interaction effect of the factors on the amount of dry matter and irrigation was not significant ($p>0.05$); While the quadratic effect of oil and chia seed mucilage on dry matter and the quadratic effect of chia seed mucilage on water content were significant ($p<0.05$).

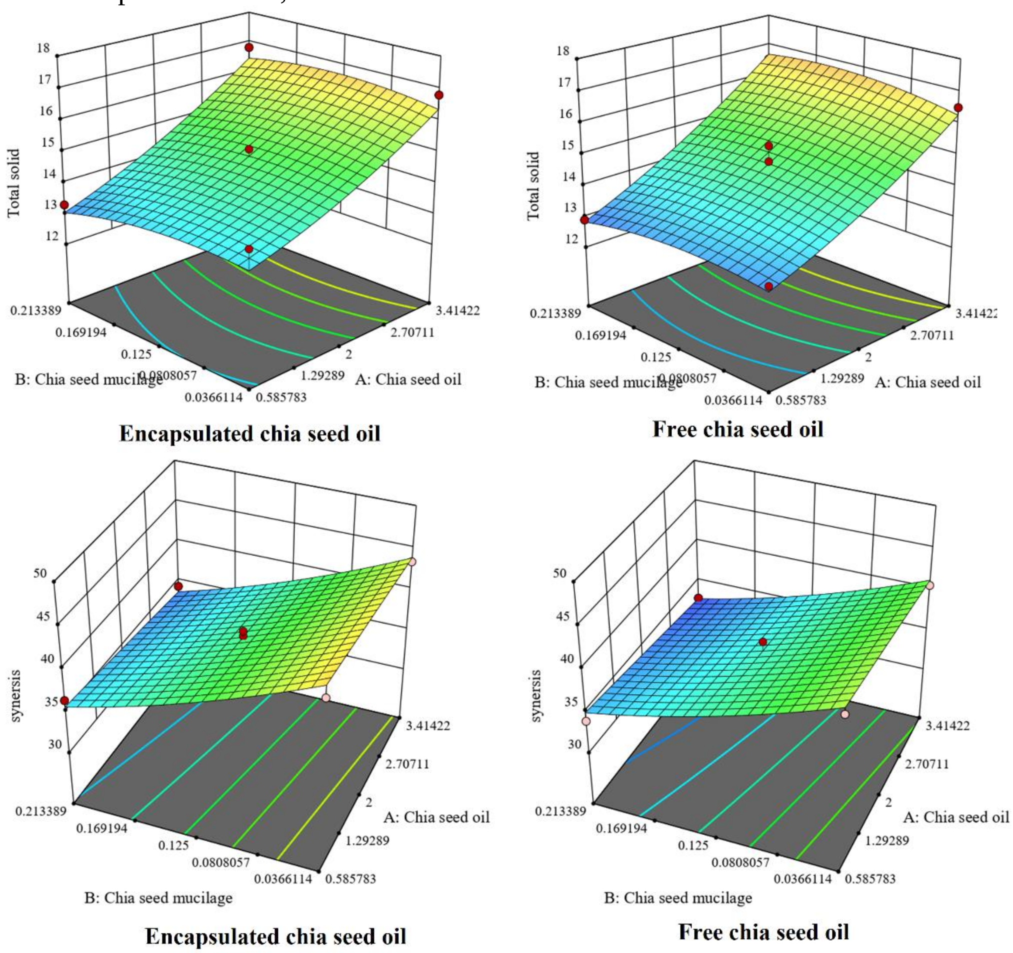

Fig 4 The change in dry matter and syneresis of yogurt vs. independent variables.

According to Figure 4, among the oil-containing samples, the microcoated samples were able to retain more water in the yogurt and thus reduce the water content of the sample. In enriched yogurt, oil causes an increase in dry matter, which probably has a favorable effect on the strength of the yogurt gel and reduces water loss in the enriched samples due to stabilizing the gel network and increasing the water binding capacity [34]. In addition, mucilage, due to its hydrophilic hydrocolloid structure, can cause a sharp decrease in the water content of yogurt. Atiq et al. (2020) also reported that the addition of any proportion of chia mucilage reduces water loss and increases the strength of yogurt samples compared to the control sample; The reason for this was the hydrocolloidal structure of chia mucilage and the absorption of water by it and the increase in the strength of the protein network of the yogurt gel [35]. According to the data analysis, the obtained quadratic polynomial model fits well with the experimental data of changes in dry matter and yogurt water content with a high explanatory factor. This result shows that the determined model has a good fit with the experimental data and it can be used to predict the quantitative performance of independent variables in yogurt watering. The influence of the independent variables of the process on the changes of the dry matter and the water content of the yogurt is shown in the disturbance diagram in Figure 5. In this figure, mucilage variable changes on the amount of dry matter, around the central point with a slight curvature, which shows the second-order effect of mucilage variable on dry matter. But due to the low curvature, the effect of the variable on the response is not strong. The variable changes of oil around the central point with an almost steep slope indicate the great effect of this factor on the changes of dry matter. The greater slope of the graph of chia seed mucilage on the amount of irrigation compared to dry matter indicates the greater effect of chia seed mucilage on the amount of irrigation.

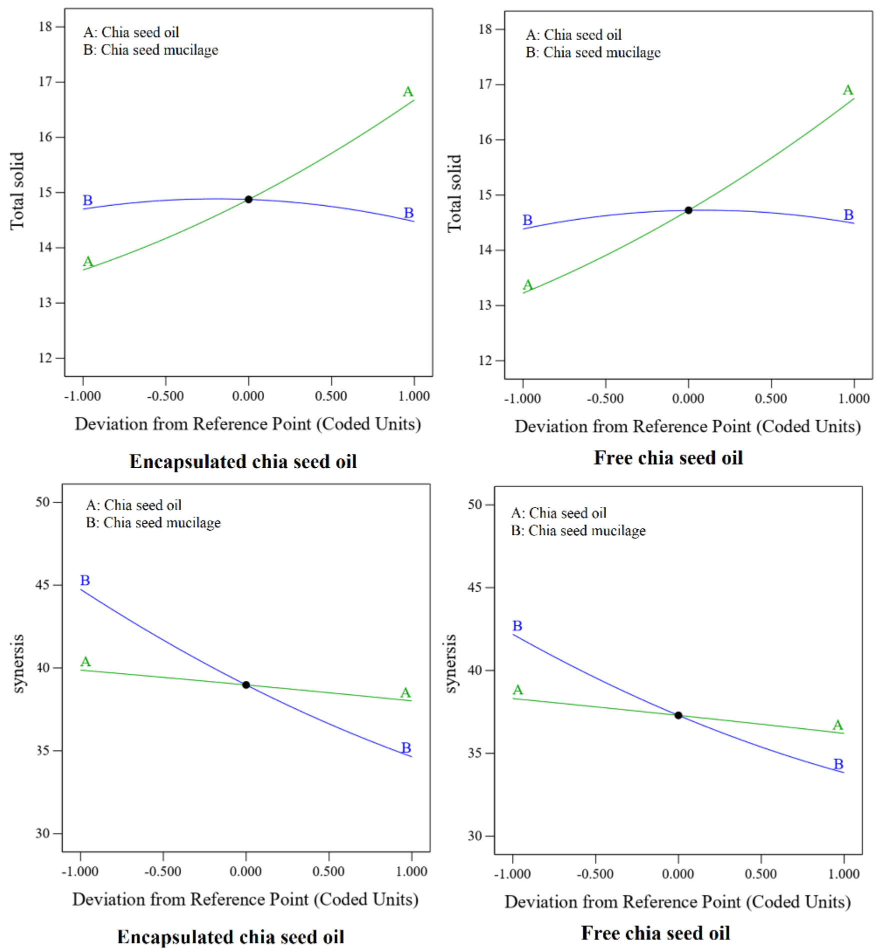

Fig 5 The perturbation plot for the change dry matter and syneresis of yogurt.

3-4-peroxide index

The results of analysis of variance showed that the amount of chia seed oil and the amount of its mucilage as independent numerical variables and the two states of free oil and microcoating have no significant effect on the change of peroxide value ($p > 0.05$). This can be due to the low peroxide value of the samples during production and the lack of effect of the microcoating process on the oxidation of chia seed oil. The amount of peroxide of all samples on the first day of production was in the range of 0.3-0.1 milliequivalent of active oxygen per kilogram of oil. It seems that the effect of the microcoating process is determined during the storage period and when the conditions for the oxidation of chia seed oil are available. Gurbanzadeh et al. (2017) also found that the microcoating process reduces the rate of peroxide increase during the storage period and its effect is not so great during the production process [36].

3-5-Color changes

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The white color of milk is due to the presence of colloidal particles such as fat globules and casein micelles [37]. Index*L Or the brightness of yogurt has a great impact on the appearance and acceptance of the product. According to the results of Figure 6, the addition of chia seed oil and the microcoating process have a significant additional effect on the index change*L Enriched yogurt samples had ($p<0.05$) and in contrast, adding chia seed mucilage to yogurt had a significant effect on the index change.L There was no microcoated sample ($p>0.05$). The results stated by Noormohammadi et al. in 2020, in the enrichment of yogurt with spirulina in a free form and micro coating with whey powder (0.5 percent), which showed that the coated samples had a whiter color, with the results of the current research on the positive effect of micro coating on increasing the whiteness It was consistent [38]. But the results of Najafi et al.'s research in 1400, in the study of yogurt enrichment with different proportions of konjak and maru gum, show a decrease in the index.L The samples were with an increase in the amount of konjak gum and a decrease in the amount of fennel seed gum, which is not consistent with the present research [29].

According to the results of Figure 6, it was found that the addition of chia seed oil and its mucilage and the mutual effect of the microcoating process and chia seed mucilage on the change of index*a (red-green) enriched yogurt samples have a significant effect ($p<0.05$); But the microcoating process alone has no effect on the change of this index ($p> 0.05$). According to the results, the increase in chia seed oil causes a decrease in the index.a became.

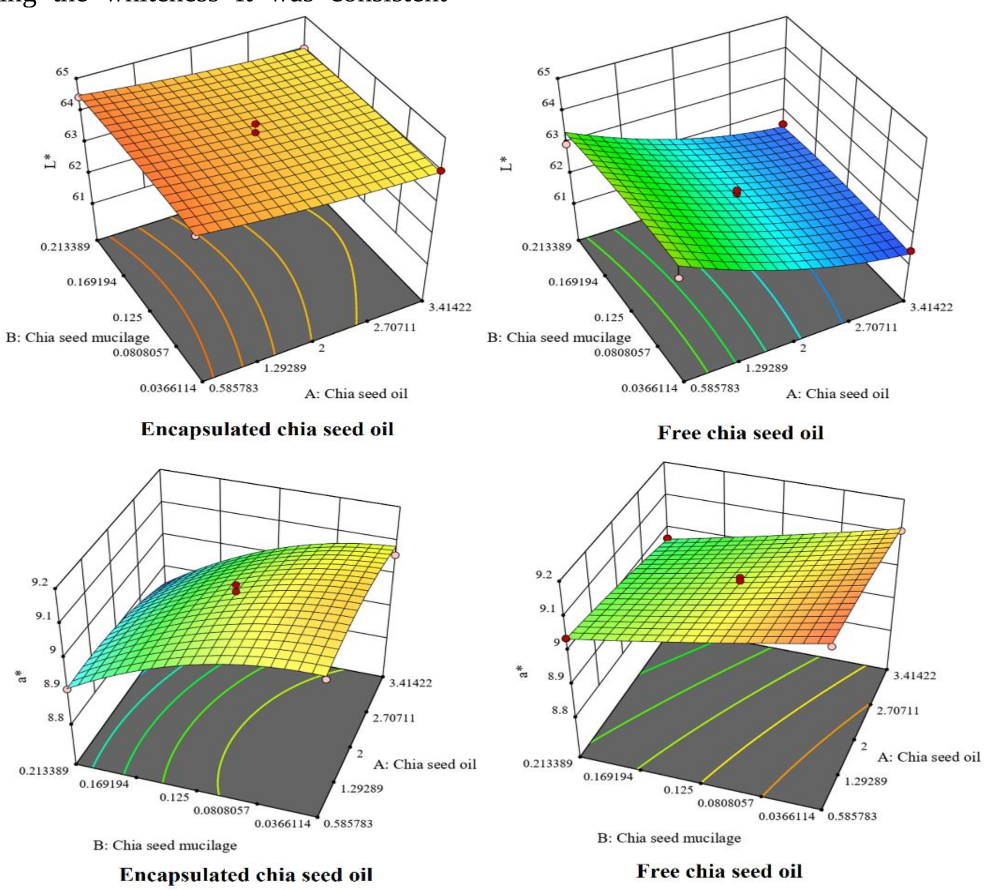

Fig 6 Chang in the $L*$ and $a*$ index of yogurt vs. independent variables.

Ghibi et al. (2018), during a research in which they investigated the addition of inulin and gum to yogurt and came to the conclusion that increasing the amount of inulin and gum in yogurt causes a decrease in the index.a is, in this research$a$ In the range of green color was one of the reasons for the decrease of this index The decrease in the water level was due to the addition of inulin and gum to the seeds [39].

According to Figure 7, the addition of chia seed oil and the coating process have a significant effect on the index change.*b* enriched yogurt samples had ($p<0.05$); However, the addition of chia seed mucilage did not have a significant effect on the change of this index ($p>0.05$). Index value **b* The micro-coated samples were in the range of -0.62 to -0.11, and the negative values indicate the blue color of the samples, and with the increase in the amount of oil in the samples without micro-coating, the index value*b* changed to higher than zero, which indicates the formation of yellow color in the samples. The lower the color changes (ΔE) of the enriched samples compared to the control sample, the better it is for the consumer. The results showed that the independent variables of oil and encapsulation process and the interrelationship between these two variables had a significant effect on the color changes of enriched yogurt ($p<0.05$), while the independent variable of mucilage and its interrelationship with two factors Other and its quadratic effects had no significant effect on the amount of color changes ($p> 0.05$). According to Figure 7, the color changes in the microcoated samples compared to the control sample had less difference compared to the free oil enriched samples. Ghaibi et al. (2018) found that the addition of gum increases the yellowness of yogurt, which is probably due to the interaction between the protein particles and the polysaccharide of the gum and as a result, destabilization of the casein micelles [39]. Previously, a significant increase ($p<0.05$) in yogurt color changes with the addition of inulin and seed gum [39], basil seed gum [41] and flax seed gum [40] was reported by other researchers.

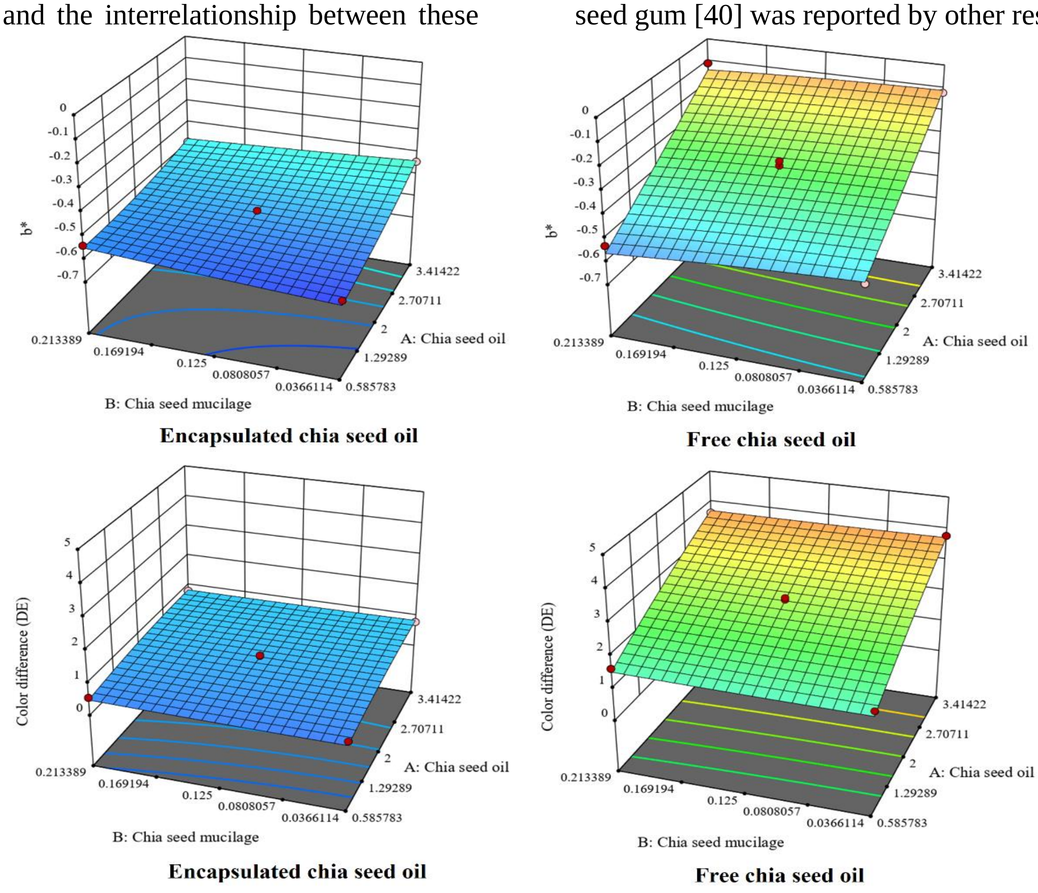

Fig 6 Chang in the *b** and *ΔE* index of yogurt vs. independent variables.

According to the data analysis, the obtained quadratic polynomial model was well matched with the experimental data of color changes and was able to explain the data with a high coefficient of explanation ($R=0.9937^2$) to predict. The closeness of experimental and predicted data of color changes also shows the success of the proposed model in predicting color changes. The effect of the independent variables of the process on the color changes by the perturbation or saturation diagram shows that in addition to the effective effect of the steep slope of the oil and chia seed mucilage factors on the response, the difference between the two images shows the great effect of the microcoating process on the color changes, so that the response is strongly It shows sensitivity to its presence or absence.

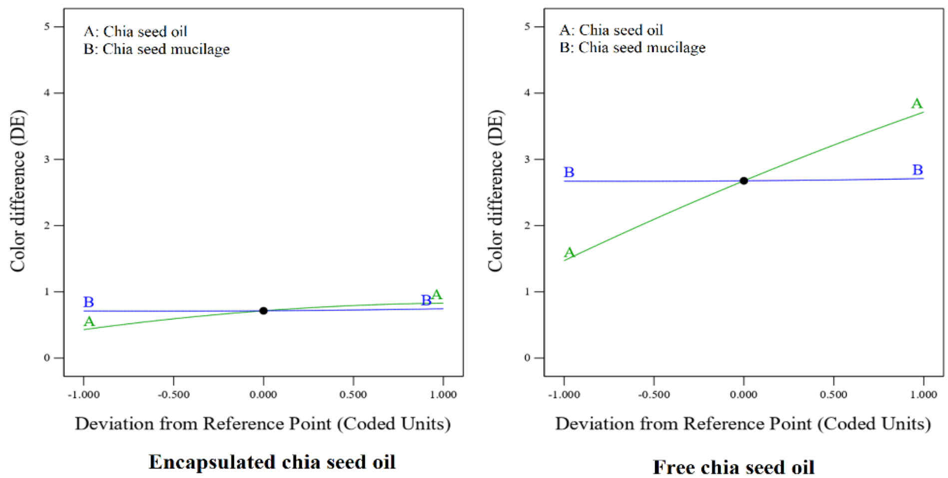

Fig 8 The perturbation plot for the color differences change of yogurt

3-6-Objective 3

Considering that dairy products are not rich in essential fatty acids, it is possible to increase the amount of these compounds in dairy products by adding compounds rich in essential fatty acids.
Dad (Nasrabadi et al., 2018). According to the results of the analysis of variance (Figure 9), the addition of chia seed oil significantly increased the amount of omega-3 in enriched yogurt samples ($p<0.05$).
Meanwhile, the addition of chia seed mucilage and the microcoating process did not have a significant effect on changing the amount of omega-3 in yogurt samples ($p>0.05$). Adding chia seed oil to yogurt can increase the amounts of oleic acid, linoleic acid, and alpha-linolenic acid, and since the amount of peroxide value has not changed much, it is likely that the amount of these fatty acids has increased in the enriched samples compared to the control sample. Food as a resourceOmega 3are considered to contain at least 0.3 grams of omega-3 per 100 grams, and foods with at least 0.6 gramsOmega 3 In 100 grams, they are considered sources with a high content of omega-3 (Kowaleski et al., 2020).The effect of the independent variables of the process on the changes of omega-3 in the disturbance diagram showed that according to the steep slope of the graph of the addition of chia seed oil, among the numerical variables studied, the amount of chia seed oil had the greatest effect on omega-3 as the studied response and the sensitivity of the response It was insignificant to other factors (Figure 9).

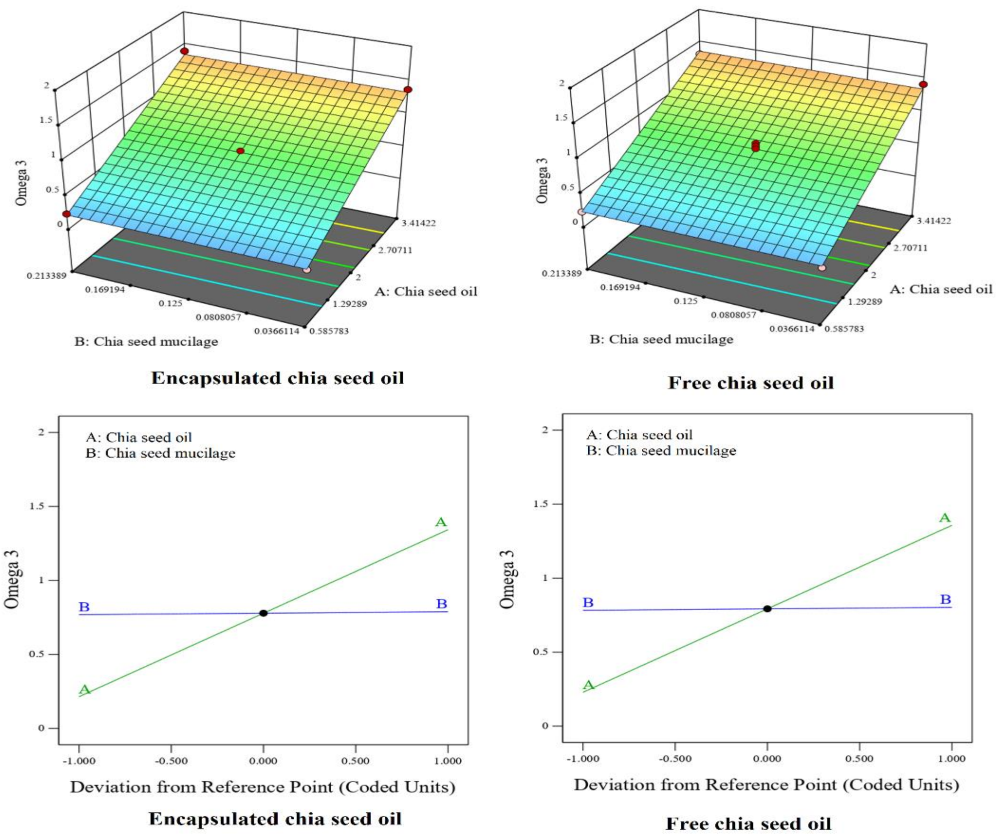

Fig 9 Change in the Omega 3 of yogurt vs. independent variables and their the perturbation plot.

Similar results were obtained by Kowalsheki et al. during the enrichment of yogurt with different ratios of chia seeds and strawberries. By examining the fatty acid profile of yogurt samples, they found that AAdding chia seeds to yogurt reduces the amount of saturated fatty acids in yogurt compared to the control sample ($p<0.05$). Also, enriched samples in terms of unsaturated fatty acids and polyunsaturated acids had a significant difference ($p<0.05$) with the control sample [8]. Other researches related to the enrichment of yogurt with compounds rich in essential fatty acids also achieved similar results. For example, Nasrabadi et al. enriched yogurt with purslane oil[35] did, they saw an increase in the amount of linolenic acid, oleic acid and linoleic acid in yogurt samples compared to the control sample; Because purslane oil, like chia seed oil, is rich in essential fatty acids [30].

7-3-Optimization

Numerical optimization of multiple responses with the response surface method and with the central composite design (CCD) with the aim of maximizing the nutritional value and simultaneously minimizing the sensory, chemical and color changes compared to the control sample and the minimum amount of watering in order to determine the optimal levels of the independent quantitative and qualitative variables of the case. was used The results of numerical optimization determined the values of independent variables for the chia seed oil factor equal to 3.41% in microcoating and for the amount of chia seed mucilage equal to 0.213%. The desirability of this optimal condition is equal to 0.896. The value of desirability is between 0 and 1, and the higher the value, the closer the selected optimal conditions are to the objectives set for optimization. Under these optimal conditions, the values of predicted and experimental responses are shown in Table 2. As can be seen in this table, there is not much difference between the experimental and predicted values.

[35]. Portulacaoleracea

Table 2 Multi-response numerical prediction results.

	Predicted values	Experimental values
pH	4.57	04.52±0.11
Acidity	0.77	0.74±0.13
Dry matter	16.43	16.51±0.25
Syneresis	33.56	34.02±0.79
Peroxide value	0.2	0.1±0.1
Color differences	0.84	0.80±0.09
Omega 3	1.33	1.39±0.12

3-7-1- Enumeration of initiator bacteria (Lactic acid)

Yogurt contains live and active lactic acid bacteria useful for human health, and according to the regulations of the National Yogurt Association for all types of yogurt, the bacteria must be active at the end of the stated shelf life. Therefore, the survival of bacteria in enriched yogurts should be investigated. The growth and survival of lactic acid bacteria is affected by many factors, including its type and species, inoculation rate, greenhouse temperature, pH, growth stimulating and inhibiting factors, storage time, concentration of metabolites and availability of nutrients [26]. The results of bacteria counting showed that the concentration of starter bacteria in both the control and optimal samples was higher than the concentration mentioned in the Codex standard.[36] (CFU/g 10^7) and the effect of microcoatings on the number of initiator bacteria is not significant compared to the control sample (p>0.05). Average number of bacteria*Streptococcus thermophilus* In control and enriched samples, it was 8.45 and 8.50 colony forming units per milliliter, respectively. Average number of bacteria*Lactobacillus bulgaricus* Also, in the control and enriched samples, the colony formation units per milliliter were 7.06 and 7.65, respectively, which did not have a significant difference (p > 0.05). Bekri et al. (2019), also investigated the effect of enriching yogurt with mint oil and tuna oil rich in omega-3, and stated that the addition of oils did not have a significant effect on the number of lactic acid bacteria.is[26].

3-7-2- Rheological properties

According to Figure 10, since the relation of shear stress in terms of shear rate in all samples is a non-linear relation, and on the other hand, because the slope of the stress-shear diagram decreases with the increase of shear rate, it can be seen that the behavior of the samples is non-Newtonian type of dilution.[37] It is with yielding tension (Herschel Balkli). The thinning behavior by cutting for yogurt samples containing chia seed mucilage and microcoatings can be explained by the spatial structure of the semi-rigid chains of the hydrocolloidal structure of the mucilage and also due to its weak gel-like structure, which causes an increase in the macromolecular engagement of the sample. This behavior is visible for most complex food samples, which is caused by their polymeric structure and high molecular weight. Diluting behavior with shear has already been reported for yogurt enriched with guar gum and chia mucilage [35]. Apparent viscosity is used in non-Newtonian fluids. Yogurt viscosity is influenced by processing methods, type of starter, type of heat treatment and the composition of the formulation that is added to it [42]. As can be seen in Figure 10, the decrease in viscosity is more severe in the lower cutting degrees, and with the increase in the cutting degree, the decrease in viscosity decreased, so that in the higher cutting degrees, the viscosity value approaches a constant value. The results of the research of Noormohammadi et al showed that the addition of micro-coating causes an increase in viscosity compared to the control sample due to water absorption by microalgae. In addition, water absorption of extracellular carbohydrates produced by spirulina increases the viscosity of yogurt. The presence of alginate and whey protein with high water absorption property causes more viscosity in samples containing microcapsules [38].

In this research, five models of Tuan, Bingham, Herschel-Bulkelley, Cason and Vokadlo were used to investigate the time-independent rheological behavior of yogurt samples. According toTable 3The results showed that the Herschel-Bulkelley model has a high efficiency (R=0.999) in the control and optimal samples^2) in describing the rheological behavior of yogurt samples. Yield stress plays a very important role

[36]. Codex Alimentarius

[37]. Shear thinning behavior

in systems with a three-dimensional network.

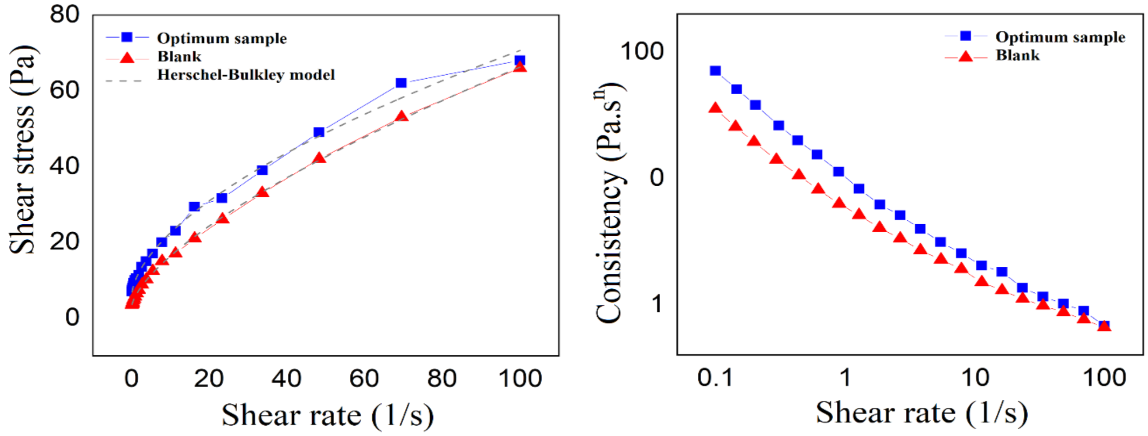

Fig 10 Shear stress-shear rate and consistency- shear plots for optimum and blank samples.

At applied shear stresses lower than the yield stress, the material in question acts like an elastic solid, and for values higher than that, like a viscous liquid. Therefore, despite the yield stress in the desired samples, the power model to fit the dataIt does not seem appropriate. In Bingham's model, by passing the yield stress, Newtonian behaviorand for this reason, this model cannot be used to determine the behavior of the desired samples. It should be noted that the closeness of the evaluation index in Vokadello and Herschel's model originates from the identical nature of these two models. The presence of yield stress for yogurt samples may be due to the large number of hydrogen bonds in their spiral structure, which leads to the creation of a stable spatial structure that resists flow. Yield stress also indicates the presence of side bonds or interactive structures in the material. Examining the data obtained from testing the optimal and control yogurt samples showed that the values of the consistency coefficient and the flow behavior index depend on the concentration of the solution, and with its increase, the value of the flow behavior index decreases and the consistency coefficient increases. The decrease in the flow behavior index with the increase in concentration can be attributed to the increase in the intermolecular conflict that causes the shear dilution behavior. Because the flow behavior index was less than one in all samples, it can be concluded that the presence of mucilage and chia seed oil did not change the fluid flow behavior. As shown in Table 3, samples containing microcoating and mucilage showed higher yield stress, which is related to stronger gel structure. Therefore, the presence of yogurt samples containing mucilage and microcoating of chia seed oil has increased the consistency coefficient and yield stress, as well as reducing the flow index.

Storage module (G`); The amount of elastic behavior and the amount of energy recovered per unit volume and drop modulus or viscous modulus (G``) represents the amount of flow behavior and the amount of wasted energy per volume unit. According to Figure 11, the predominance of the elastic property indicates the existence of a network structure and gel behavior. Both elastic and viscous modulus are dependent on frequency and their values have increased with increasing frequency. The addition of chia seed oil microcoating and chia seed mucilage to yogurt increased the storage and loss modulus compared to the control sample, and the higher storage and loss modulus indicates the gel-like behavior of this system. The non-crossing of the elastic and viscous graphs shows that no phase change occurred in the samples in the tested time range and always G` from G`` is bigger; Therefore, the stability of all samples is confirmed by this test [43].

Table 4 Shear stress-shear rate parameters and consistency- shear plots for optimum and blank samples.

	Blank sample	Optimum sample

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	Power-law model	
K (Pa.s^n)	4.58±0.29	8.72±0.61
n	0.57±0.016	0.44±0.018
R^2	0.993	0.982
RMSE	1.51	2.56
	Herschel-Bulkley model	
t_0 (well)	3.04±0.17	5.75±0.69
K (Pa.s^n)	2.79±0.10	4.31±0.49
n	0.67±0.008	0.58±0.02
R^2	0.999	0.995
RMSE	0.38	1.1
	Bingham model	
t_0 (well)	6.71±0.82	11.41±1.19
m^p (Pa.s^n)	0.65±0.02	0.66±0.03
R^2	0.971	0.943
RMSE	3.13	4.54
	Vocadlo model	
t_0 (Well)	3.74±0.21	7.22±0.55
K (Pa.s^n)	7.37±0.64	35.16±9.20
n	0.63±0.008	0.51±0.01
R^2	0.999	0.995
RMSE	0.51	1.19
	Casson model	
t_0 (Well)	3.79±2.55	5.39±1.00
K (Pa.s^n)	109.12±36.9	205.19±39.85
R^2	0.660	0.805
RMSE	10.76	8.45

Figure 11 shows that the values of the elastic modulus of the samples in the applied frequency range are higher than the viscous modulus, so it can be said that all the samples have the ability to form a weak gel, which increases with the addition of microcoating and mucilage. With the addition of mucilage, which is classified as a neutral hydrocolloid, non-electrostatic interactions occur between the chia seed mucilage and the casein aggregates, which increases the elastic properties of yogurt; Probably, this hydrocolloid strengthens the casein network by connecting with the positive charges on the surface of casein micelles and increases the elastic modulus.

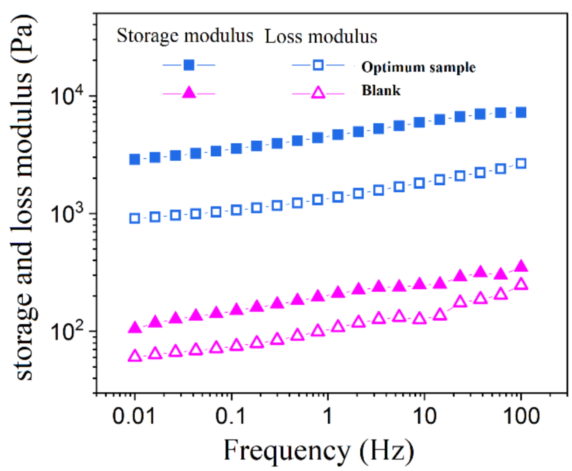

Fig 11 Storage and loss modulus-frequency plot in optimum and blank samples.

4- General conclusion

The present study was conducted to investigate the possibility of enriching yogurt with chia seed oil and mucilage. First, in order to increase the oxidative stability of chia seed oil, its oil was

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microencapsulated by emulsification-gelation method in alginate coating and chia seed mucilage. The results of the evaluation of the microcoating process showed that the efficiency of the emulsification method for microcoating of chia seed oil is 89.8±0.24%. In other words, nearly 90% of the added chia seed oil was enclosed in the microcoatings, and only 10% of it remained uncoated. The results of scanning electron microscope images showed the successful formation of sodium alginate-chia seed mucilage microcoating with a uniform shell structure. The results obtained for peroxide number and encapsulation efficiency show that microencapsulation of chia seed oil by emulsification method in alginate coating and chia seed mucilage is an effective strategy to prevent oxidation of chia seed oil in enriched yogurt. The results obtained from the physical characteristics showed that the enrichment caused a decrease in water retention, an increase in the storage capacity and an increase in the viscosity of yogurt. The production of yogurt containing the microcoating of chia seed oil and its mucilage, which is rich in omega-3, can be considered as a health-enhancing nutritious food.

5- Resources

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غنی‌سازی ماست با روغن درون‌پوشانی‌شده و موسیلاژ دانه چیا: بهینه‌سازی و ارزیابی ویژگی‌های فیزیکی‌وشیمیایی و رئولوژیکی

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چکیده

پژوهش حاضر با هدف بهینه‌سازی فرمولاسیون ماست غنی‌شده با روغن و موسیلاژ دانه چیا به‌منظور به حداکثر رساندن میزان اسید چرب آلفا-لینولنیک (امگا ۳) و حداقل تغییرات در ویژگی‌های فیزیکی‌وشیمیایی (pH، اسیدیته قابل‌تیتر، آب‌اندازی، ماده خشک، پراکسید و رنگ) نسبت به نمونه شاهد بود. ابتدا به منظور افزایش پایداری اکسایشی، روغن دانه چیا با استفاده از آلژینات سدیم و موسیلاژ دانه چیا و به کمک روش امولسیون‌سازی-ژلاسیون داخلی ریزپوشانی گردید. سپس نمونه‌های ماست حاوی ۰/۲۵-۰ درصد موسیلاژ دانه چیا و ٤-۰ درصد روغن دانه چیا به دو صورت آزاد و ریزپوشانی‌شده تولید گردید. نمونه فاقد روغن و موسیلاژ دانه چیا به‌عنوان نمونه شاهد می‌باشد. نتایج تصاویر میکروسکوپ الکترونی روبشی نشان‌دهنده تشکیل موفقیت‌آمیز ریزپوشش آلژینات سدیم- موسیلاژ دانه چیا با ساختار پوسته یکنواخت بود. نتایج بدست آمده از ویژگی‌های فیزیکی‌وشیمیایی نشان داد که غنی‌سازی سبب کاهش معنی‌دار آب‌اندازی ($p<۰/۰۵$) و افزایش معنی‌دار ($p<۰/۰۵$) ظرفیت نگهداری و ویسکوزیته ماست گردید. به علاوه ماست حاوی ۳/٤۱ درصد روغن دانه چیا به‌صورت ریزپوشانی‌شده و ۰/۲۱۳ درصد موسیلاژ دانه چیا، به عنوان نمونه بهینه تعیین شد. نتایج نشان داد که نمونه بهینه از نظر تعداد باکتری‌های آغازگر نیز تفاوت معنی‌داری با نمونه شاهد ندارد ($p>۰/۰۵$). بافت ماست‌ها، جامد ویسکوالاستیک تشخیص داده شد و در تمام نمونه‌ها همواره در کل محدوده فرکانسی، مدول ذخیره (G') از مدول افت (G'') بیشتر بود. شاخص رفتار جریان در کلیه نمونه‌ها کمتر از یک بود که نشان دهنده رفتار غیرنیوتنی (هرشل بالکلی) نمونه‌ها می‌باشد. مدل هرشل بالکلی در هردو نمونه بیشترین ضریب تبیین ($R^2 =۰/۹۹$) و کمترین ریشه میانگین مربعات خطا را دارا بود که نشان دهنده مناسب بودن این مدل برای مدل‌سازی رفتار رئولوژیکی نمونه‌ها است.