

Study the Effect of Adding Iron Salts on the Physiochemical and Sensory Properties of Yoghurt

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Abstract

Yoghurt was manufactured by adding ferrous sulfate (T1) before heat treatment, (T2) after heat treatment, and adding ferrous chloride (T3) before heat treatment and (T4) after heat treatment as well as the control sample separately, the physiochemical tests were performed for Yoghurt the above treatments a day after manufacturing and after storage for 21 days at a temperature of 5 ± 1 ° C, and our results were: There were no significant differences in pH values after one day of manufacturing for all treatments, either during storage for 21 days at a temperature of 5 ± 1 ° C, a gradual decrease was observed in them, either with respect to the percentage of total acidity (TA), it was close to all treatments of the different Yoghurt after one day of manufacturing. either when storing, it was observed that it increased for all transactions. The concentrations (2.5, 5)Mm played a role in improving the physicochemical properties of Yoghurt, such as reduced Syneresis, increased Water holding capacity, and improved viscosity and hardness compared to other samples. As for the high concentrations (7.5, 10)Mm, it had a negative effect on the rheological properties of the Yoghurt.

Keywords: adding iron salts, physiochemical, sensory properties, Yoghurt

Introduction

Iron is necessary in the human diet, and when there is an imbalance or deficiency in one or more of the essential nutrients, or a deficiency or an increase in the intake of calories, it leads to the occurrence of malnutrition ¹. Iron plays an important physiological and chemical role for most Living organisms, including animals and humans alike, it is present in the structure of the Cytochrome, enzymes, hemoglobin and myoglobin ² Milk and other dairy products such as Yoghurt and cheese are close to a nutritionally ideal food that contains all the nutrients needed for newborns, adults and the elderly, and a good source of protein, fats and carbohydrates as well as vitamins, calcium and phosphorous. However, it is generally a poor source of trace elements such as Iron, zinc, manganese and copper ³ In addition to the nutritional importance, it is known that fortifying the Yoghurt with iron can take place a chemical reaction with the various food components, which leads to changes in the physiochemical properties important to the quality properties of the Yoghurt. In order to devise

the best ways to compensate for the deficiency in the iron element and to meet the daily need for it in our country and to improve the physiochemical and sensory properties of Yoghurt

Materials and Methods

A. Milk sources

used dried fat-free cow's milk (1%) fat, which was obtained from local markets from Baghdad and the product is from (Nijkerk) company

B. Yoghurt preparation and physiochemical analysis

Yoghurt manufacturing

The Yoghurt was manufactured in this study according to the method used (Jayalaita et, 2012). (4) Salt was added to the milk before heat treatment and after heat treatment.

The dried cows' milk produced from the company (Nijkerk) was recovered into the proportion of magnetic

materials 13% with a weight of 130 g and the volume was completed with water to 1 liter. (5 liters were prepared), then the prepared quantity was divided into five sections, the first section without using the use in the manufacture of Yoghurt for the control treatment, while the other four sections were added to each liter of them ferrous chloride at a concentration of (10, 7.5, 5, 2.5) Mm / Liter of milk and mixing well, then the milk was subjected to a heat treatment at a temperature of 90 ° C for 10 minutes, then cooled to a temperature of 42 ° C. It was packed in 125 ml containers and incubated at a temperature of 42 ± 2 ° C until the coagulation was complete, within 4-5 hours, the pH was reduced to 6.4, then it was removed from the incubator for inflation and kept at a temperature (5 ± 1) until the necessary tests. After 1, 7, 14, and 21 days of processing, this process was repeated in the manufacture of samples of Yoghurt with ferrous sulfate added. The first practical process in the refining process where iron salts were added after heat treatment.].

pH determination

The pH of the Yoghurt samples was estimated after 1, 7, 14, 21 days of processing by applying a sensor pH meter type HQ 411 d (Model 211) German origin, directly in the sample of the Yoghurt

Titrateable acidity determination

Titrateable acidity of yoghurt samples was estimated according to A.O.A.C. (2008) ⁵ by taking 9 ml of milk (9 grams of yogurt) in a beaker and adding to it a few drops of phenolphthalein reagent at a concentration of 1% and then powdering it with sodium hydroxide (NaOH) at a concentration of 0.1 nm until the pink color appears, and the total acidity percentage was calculated based on Lactic acid according to the following equation:

$$\text{Total acidity} = \frac{\text{volume of NaOH 0.1 used} \times 0.09}{\text{weight of the sample}} = \% \times 100$$

Viscosity determination

The viscosity of the Yoghurt samples was determination at a temperature of 10 ° C after 1, 7, 14 and 21 days of refrigerated storage at a temperature of (5 ± 1) ° C using the Brookfield viscometer prepared

by Brookfield Engineering Lab Inc., Stoughton, Mass. As he used the axial spindle No. 4, with a number of revolutions of 10 revolutions / minute, with a volume of 125 ml, according to what was mentioned ⁶.

C. Syneresis determination

Syneresis was determined according to the procedure described by ⁷. A cup of the set yoghurt was removed from refrigerator at 5oC. A needle connected to syringe was used to withdraw the liquid whey from the surface of the sample and the cup of fermented skim was weight again. The process lasted for less than 10s to avoid further leakage of whey from

the Yoghurt.

D. Water-holding capacity determination

Water-holding capacity (WHC) of yoghurt was determined as described by ⁸ Briefly, 10 g of yoghurt was centrifuged at 5000xg for 10 min at 5oC. The resulting supernatant was carefully weighted to determine the amount of excluded water,

$$\text{WHC \%} = [1 - (w2 / w1)] \times 100$$

[w1: weight of yoghurt used, and w2: weight of whey after centrifugation].

E. Texture determination

The evaluation of textural properties was conducted using a texture analyzer CT3(4500), Brookfield engineering lab .

The hardness of samples were measured and the operation conditions were an artificial plastic cylinder (20 mm in diameter) was inserted into each product to a depth of 20 mm with 5.0g trigger and speed of 1 mm/s .

F . SEM scanning electron microscopy test

For SEM, sections 3 × 3 × 3 mm were excised from the yoghurt, approximately 1 cm below the surface and were fixed in 2.5% glutaraldehyde solution in phosphate buffer at pH of 7.4 for 1 days (9). The section were then cut into prisms 3 × 3 × 3 mm, dehydrated in a graded ethanol series (10,20,30, 40, 50, 60,70, 80, 90, and 100%), For a period of 30 minutes per concentration. After the completion of drying the samples were frozen, after which the frozen samples were permanently dried

by the (ALPHA 1–4 CHRIST) lyophilizer.

The samples were placed in aluminum containers of a scanning electron microscope and coated with a layer of gold and bilenium water with a thickness of 5 nm. Images were acquired with a voltage of 1.4 kV and different enlargement powers.

G. Sensory evaluation

Sensory evaluation of Yoghurt milk fortified with iron salts was performed after 1, 7, 14 and 21 days of manufacture. The samples were presented in small cups of (125 ml) capacity and were evaluated by a number of professors at the College of Food Sciences / Al-Qasim Al-Green University. The samples were evaluated in terms of flavor, texture, appearance and color according to the sensory evaluation form previously developed. By¹⁰.

Statistical Analysis

All possible comparisons among the means were carried out by using Least Significant Difference (LSD) test at the significant level of 0.05 after they show their significant in the general test

Results and Discussions

The effect of adding iron salts on the pH of Yoghurt:

The pH is a major factor in determining the amount of development of the casein network responsible for Yoghurt formation ^{11,12}.

The results shown in Fig. (4-1)A, B, C, D the pH of Yoghurt with added iron salts for (T1), (T2), (T3), and (T4) respectively.

The results of the statistical analysis of the above factors indicate that there are no significant differences (P <0.05) between the pH values of the control sample and the samples with iron added. These results show that the addition of iron before and after the heat treatment has no effect on the pH of the Yoghurt, as it does not affect the starter culture bacteria, and this is consistent with what was found ¹³ which showed that there was no significant effect of adding ferrous sulfate to Yoghurt e on the numbers of bacteria. The beginning, and by advancing the storage period at a temperature of ± 5 ° C. We notice a gradual decrease in the pH values of all samples, and this is due to the continued activity of the starter culture bacteria in converting lactose into lactic acid ¹².

We also note that the pH values of (T4) were slightly higher than treatment (T3) and this is due to the effect of the double heat treatment that led to the expulsion of a greater amount of CO₂ and also the removal of amine groups from the protein, which leads to the formation of ammonia that raises the pH of the Yoghurt (Al-Saadi et al2012).(14).

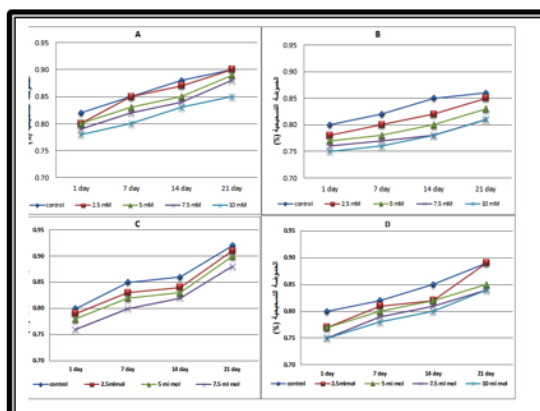


Figure (4-2) The effect of adding iron salts on the acidity of the yoghurt A (T1) Addition of ferrous sulfate before the heat B (T2) Addition of ferrous sulfate after the heat treatment C (T3) addition of ferrous chloride before the heat treatment D (T4) Addition of ferrous chloride after heat treatment

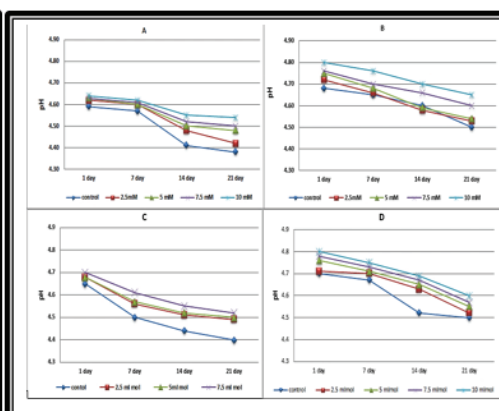


Figure (4-1) The effect of adding iron salts on the pH of the yoghurt A (T1) Addition of ferrous sulfate before the heat B (T2) Addition of ferrous sulfate after the heat treatment C (T3) addition of ferrous chloride before the heat treatment D (T4) Addition of ferrous chloride after heat treatment

Through the figure (A, B, C, D), we find the pH values of the samples with added iron salts, a non-significant increase compared to the control samples, and the reason for this is due to the possibility that these salts work to form buffer solutions that reduce the changes in pH values.

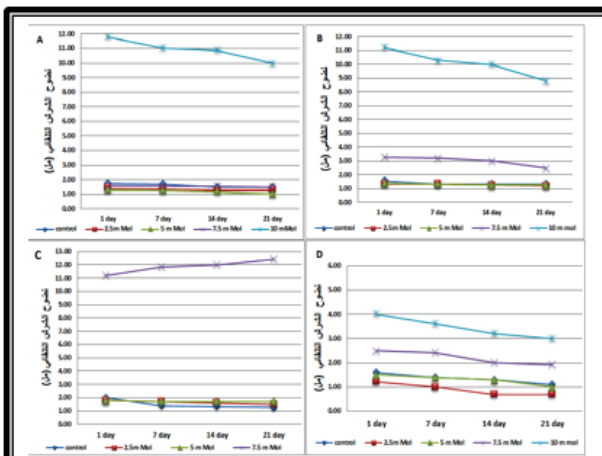


Figure (4-4) The effect of adding iron salts on the Syneresis of the yoghurt A (T1) Addition of ferrous sulfate before the heat B (T2) Addition of ferrous sulfate after the heat treatment C (T3) addition of ferrous chloride before the heat treatment D (T4) Addition of ferrous chloride after heat treatment

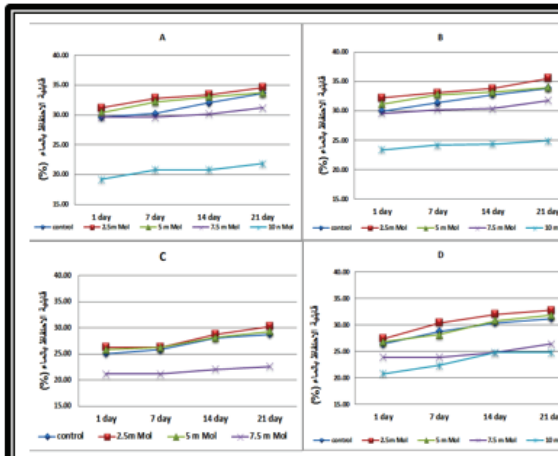


Figure (4-5) The effect of adding iron salts on the (WHC) of the yoghurt A (T1) Addition of ferrous sulfate before the heat B (T2) Addition of ferrous sulfate after the heat treatment C (T3) addition of ferrous chloride before the heat treatment D (T4) Addition of ferrous chloride after heat treatment

The effect of adding iron salts on the Yoghurt acidity:

Figure (4-2) (A, B, C, D) shows the values of the acidity based on the lactic acid of the Yoghurt with iron salts added for the treatments (T1), (T2), (T3), (T4) respectively.

The results of the statistical analysis of the above factors showed that there were no significant differences (P <0.05) between the restorative acidity values of the control sample and the samples with iron added during the storage period due to the lack of effect of adding iron before and after the heat treatment on the total acidity of the Yoghurt and this is due to its lack of influence on the starting bacteria Responsible for the production of lactic acid (15).

As for after storage at a temperature of $5 \pm 1 \text{ }^\circ\text{C}$ for a period of 21 days, an increase in the acidity values of the control sample and the samples added to it (2.5, 5, 7.5 and 10) Mm of iron are observed, and these results are consistent with (Ziena et al.2019)(15) referred to. It indicated an increase in the overall acidity of the Yoghurt e fortified with ferrous sulfate after storage due to the increase in the activity of the starting bacteria and its persistence in the production of lactic acid and its non-

effect on the starting bacteria.

From Figure (4-2) A, B, C, D, we find the existence of non-significant differences in the parameters to which iron was added after the heat treatment (D, B), as their values were less than the treatments that were added to iron before the heat treatment (C, A). This is due to the effect of double heat treatment on the pH of the Yoghurt (14)

The effect of adding iron salts on Syneresis from Yoghurt:

The Fig. (4-4)A, B, C, D showed the level of Syneresis of Yoghurt with added iron salts for the treatments (T1), (T2), (T3) and (T4), respectively, significant differences were observed In their values after storage for one day at $5 \pm 1 \text{ }^\circ\text{C}$.

The levels of Syneresis after one day of storage. For the treatment (T1) shown in Figure (4-4) A for the control sample was (1.76) ml, and the samples with added (2.5, 5, 7.5 and 10)Mm of ferrous sulfate were (1.42, 1.32, 1.60 and 11.80) ml, respectively.

And for the treatment (T3) in Fig. (4-4) C, the control sample was (2.00) ml, and the samples added to it (2.5, 5, and 7.5) Mm ferrous chloride before the heat

treatment were (1.80, 1.82 and 11.20) ml, respectively. The results shown the addition of ferrous chloride after heat treatment at concentrations of (2.5 and 5) Mm reduced the levels of spontaneous exudation compared to the control sample, this is consistent agrees with (Sadiq & Doush, 2019)(20), where addition of coated and non-coated iron improved the The Syneresis from Yoghurt due to the iron-protein bonds. As for the concentration of 10 Mm, it was the highest in the level of Syneresis. This is due to the weakening of the high concentrations of the protein network due to the precipitation of a part of the casein (17).

The effect of adding iron salts on the water holding capacity of the Yoghurt:

The (WHC) depends on the proteins present in the food, and the type of amino acids that are included in the natural protein composition, And the amount of polarity of amino acids and the extent of hydrophobicity or hydrophilicity ((22

The Fig. (4-5)A, B, C, D showed the (WHC) of Yoghurt with added iron salts (T1), (T2), (T3) and (T4), respectively.

The (WHC) after one day of storage. at 5 ± 1 ° C. For the treatment (T1) in Fig. (4-5) A for the control sample was 029.7% and for the samples with added (2.5, 5, 7.5 and 10) Mm ferrous sulfate before the heat treatment were (31.20, 30.40, 29.60 and 19.20)%, respectively.

We find that the addition of iron in this treatment at concentrations of (2.5 and 5)Mm improved the (WHC) for Yoghurt compared to the control sample, although the differences are not significant, but the reason is that the iron formed bonds with the protein, which confined a larger amount of water inside it (21) compared to the concentrations (7.5 and 10)Mm, whose ability to preserve water was less than the control treatment, since the higher concentrations affected the structural composition of the Yoghurt due to the reduction of the charge on the caseins, thus reducing its ability to bind with water. (23).

And for the treatment of (T2),in Fig (4-5) B, the control sample was 30.00%, and the samples with added (2.5, 5, 7.5 and 10) Mm ferrous sulfate after the heat treatment were (32.20, 31.20, 29.60 and 23.40)%,

respectively. .

The results indicate that the addition of ferrous sulfate after heat treatment at concentrations of (2.5 and 5)Mm improved the (WHC) of Yoghurt compared to the control sample. As the concentrations (7.5 and 10) Mm , their ability to (WHC) was less than that of the control treatment, since the high concentrations affected the structural composition of the Yoghurt (23).

The treatment (T3) in Fig. (4-5) C for the control sample was 25.04%, and for the samples with (2.5, 5 and 7.5) Mm ferrous chloride added before the heat treatment were (26.28, 25.80 and 21.20)%, respectively.

We find that adding ferrous chloride before heat treatment with (2.5 and 5)Mm improved the (WHC) of Yoghurt compared to the control sample, this is result consistent with (19) who founded the ability of the protein network associated with iron increase binding Water and that the link increases with the increase in the amounts of added iron, to certain concentrations, the protein network begins to collapse, and this is happened in the concentration of 7.5 Mm in this treatment .

For the treatment (T4), in Fig. (4-5) D, the control sample was (26.40)%, and the samples to which were added (2.5, 5, 7.5 and 10)Mm ferrous chloride after heat treatment were (27.40, 26.80, 23.92 and 20.80)% respectively.

These results indicate the ability of iron chloride added after heat treatment at concentrations of (2.5 and 5) Mm increase the (WHC) of the Yoghurt.

After storing at 5 ± 1 ° C for 21 days, we find an increase in the percentage of (WHC). It was for (T1) (34.60, 33.70, 31.20, 21.80)%, (T2) (35.60, 34.00, 31.80, 24.90)%, (T3) (30.24, 29.28, 21.61) % (T4) (32.80, 31.80, 26.40 and 24.80) %, respectively, and this is due to the increase in strength, and the number of bonds that iron made with milk proteins, this result is consistent with (21) found, increase in (WHC) of Yoghurt treated with iron after storage is due to the increase in the number and strength of the bonds formed by iron with protein.

Through Fig. (4-5) D, C, B, A, shone that the (WHC) of Yoghurt fortified with ferrous sulfate was higher than the samples fortified with ferrous chloride and for both

cases of addition before and after the heat treatment. We also find that the percentage of retention ability in the figures (D, B) were higher than (C, A) due to the role of double heat treatment in increasing the denaturation of the whey proteins and increasing the binding capacity of a greater amount of iron (24).

The effect of adding iron salts on the viscosity of Yoghurt:

The figure (4-3) A,B,C,D showed the viscosity of the Yoghurt with iron salts added for the (T1), (T2), (T3) and (T4), respectively, in which significant differences were observed in their values after and after storage for one day at a temperature of $5 \pm 1^\circ \text{C}$, it was For the treatment (T1) in Fig. (4-3) A to the control sample (2800) centipoise and the samples with added (2.5, 5, 7.5 and 10)Mm of ferrous sulfate before the heat treatment were (2940, 2980, 2400 and 680) centipoise, respectively.

We find that adding ferrous sulfate before the heat treatment with (2.5 and 5) Mm improved the viscosity property and its values were higher than the control sample values due to the ability of iron to extend bridges between casein particles, which leads to an increase in its size and thus improving the viscosity. These results are in line with The result reached by (15), as for the high concentrations, they had a negative effect, as the concentrations (7.5 and 10) Mm reduced the viscosity values compared to the control sample, as the addition of iron above the saturation level of casein leads to its precipitation and thus the viscosity values decrease, this is in agreement. With (23), where they stated that adding iron to skim milk occurred protein precipitation when the concentration of iron added to milk was about (8) Mm.

And the treatment (T2), showed in Fig. (4-3) B, the control sample was (4300) and the samples with ferrous sulfate added after heat treatment (2.5, 5, 7.5 and 10) Mm were (4390, 3370, 1990 and 300) centipedes,

respectively.

We find that the concentration of 2.5 Mm of ferrous sulfate added after the heat treatment improved the viscosity, while the viscosity values of the concentrations higher than 2.5 Mm were decreased compared to the control sample. This is due to the competition of iron-added calcium from binding to casein, which caused the small size of casein particles, this is in agreement with . (Mittal et al. 2015)(24) indicated that when calcium is depleted and iron is added at concentrations of (5, 10, 20) Mm, the added iron is responsible for the high ability to bind to proteins in milk instead of calcium.

The effect of adding iron salts on the hardness of the Yoghurt

The above results show that the addition of ferrous in this treatment improved the hardness of the Yoghurt, especially the concentrations (2.5 and 5) Mm. As for the high concentration of iron (7.5)Mm, it led to a decrease in the level of hardness because the addition of iron to the protein above a certain concentration, when The binding sites are saturated, leading to protein precipitation. This is in agreement with indicated by (25).

The treatment (T4) showed in Fig. (4-6) D, to the control sample was (98.60) gm., and for the samples with (2.5, 5, 7.5 and 10) Mm of ferrous chloride added after the heat treatment were (101.30, 81.70, 67.60 and 65.10) gm., respectively.

We find the existence of an effect of adding ferrous chloride after heat treatment positively on the hardness values of Yoghurt at a concentration of only 2.5 Mm compared to the treatment (T3) that affected the concentrations (2.5 and 5)Mm. As for the higher concentrations, it negatively affected the protein structure compared to the control sample due to the effect of the treatment. Thermocouple increases the iron binding and thus the effect on the protein network.

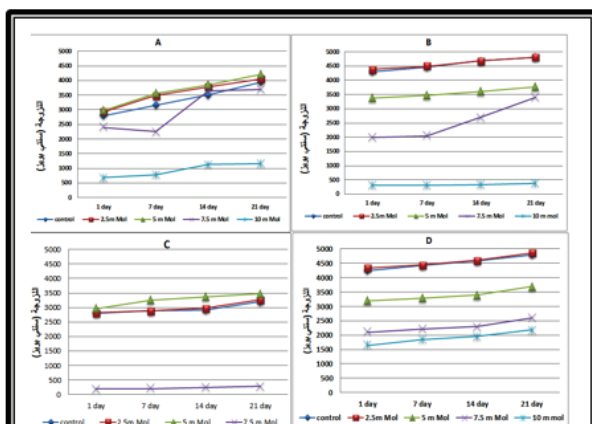


Figure (4-5) The effect of adding iron salts on the **viscosity** of the yoghurt A (T1) Addition of ferrous sulfate before the heat B (T2) Addition of ferrous sulfate after the heat treatment C (T3) addition of ferrous chloride before the heat treatment D (T4) Addition of ferrous chloride after heat treatment

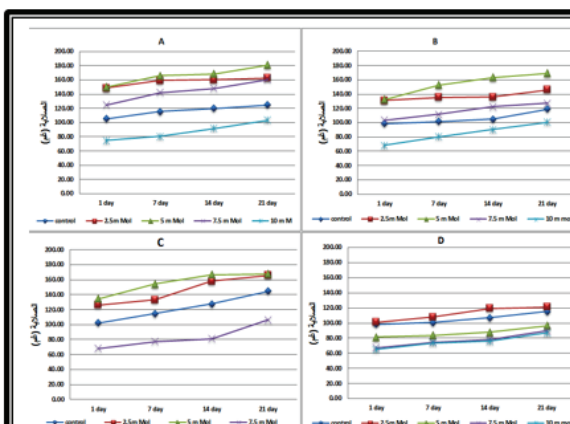


Figure (4-6) The effect of adding iron salts on the **hardness** of the yoghurt A (T1) Addition of ferrous sulfate before the heat B (T2) Addition of ferrous sulfate after the heat treatment C (T3) addition of ferrous chloride before the heat treatment D (T4) Addition of ferrous chloride after heat treatment

. Thermocouple increases the iron binding and thus the effect on the protein network.

However, after 21 days of storage at a temperature of $5 \pm 1\text{C}^\circ$, the hardness values of all samples increased, reaching (T1) treatment (162.40, 181.10, 160.90, 103.50) gm, (T2) (145.90, 169.30, 127.20 and 100.60) gm, (T3) (165.80, 168.00 and 106.60) gm, (T4) (121.40, 96.40, 89.70 and 86.80) gm. Accordingly, as a result of protein aggregation and the bonds formed by iron that increase the cohesion within the protein network and increase its hardness (28).

From Fig. (4-6) D, C, B, A, we note that there are differences in the parameters to which iron was added after the heat treatment (D, B). The hardness values were less than the treatments that were added to iron before the heat treatment (C, A). This is due to the effect of heat treatment on increasing the accumulation of the denitrified whey proteins, and this is consistent with what was found by (W. Gustaw, 2007)(29).

Conclusions

The possibility of manufacturing yoghurt fortified with iron salts at certain concentrations without any change in composition compared with the yoghurt for the control treatment. The addition of iron salts to the yoghurt did not lead to significant differences in the pH values compared to the control treatment. Concentrations (2.5, 5) Mm of iron salts, improved the physicochemical properties of yoghurt in terms of reduced spontaneous perfusion, increased water retention, and improved

viscosity and hardness compared to other samples. The high concentrations of iron salts (7.5, 10)Mm had a negative effect on the rheological and sensory characteristics of the yoghurt. By SEM scanning electron microscope, the effect of adding iron salts was observed on the protein network binding and improving the yoghurt properties.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Dairy Science and Technology and all experiments were carried out in accordance with approved guidelines.

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