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Whey Protein-Based Nanoformulation Loaded with *Moringa Oleifera* Extract, and Functionalized with Folic Acid for Studying its Effect on Yogurt Properties

Nidaa Owayez Abbas Al-Shabbani¹, Jasim M. S. Al-Saadi², Sharafaldin Al-Musawi³

1,2-Department of Food Science and Technology, College of Food Sciences, Al-Qasim Green University, Babylon, Iraq.

3-Department of Dairy Science and Technology, College of Food Sciences, Al-Qasim Green University, Babylon, Iraq

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ABSTRACT

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*Corresponding Author E-Mail:

nidaaishbany@fosci.uoqasim.edu.iq

Recently, there has been a strong interest in nanoparticles (NPs) and their use in a wide range of food applications due to their possession of a set of unique physical and chemical properties. The texture, stability, and nutritional value of dairy yoghurt can be enhanced using whey protein nanoformulation, resulting in improved consumer satisfaction. The goal of this work was to design and synthesize a nanoformulation of folate (FA) functionalized with whey protein isolate (WPI) and loaded with moringa (MOR) extract, and to utilize it in the development of various yoghurt characteristics. WPI-MOR-FA nanocomposite was analyzed by dynamic light scattering (DLS), scanning electron microscope (SEM), Transmission Electron Microscope (TEM), and Fourier transform infrared (FTIR) spectroscopy. They added these NPs to skim milk and produced yoghurt from it. Under varying conditions, the influence of this addition on the chemical, physical and sensory characteristics of yoghurt was examined. The WPI-MOR-FA NPs had desirable zeta potential, size and polydispersity. Also, the microscopic analyses meant the round shape and reduced aggregation. The FTIR test proved the accuracy of the synthesized NPs. The MOR loading and encapsulation efficiency were within appropriate ranges, and the release study demonstrated the liberation of MOR within the expected range. Sensory evaluation results indicated that consumers accepted the yogurt product to which the nanocomposite was added at all concentrations, as it tasted better and was whiter. These findings highlight the potential of WPI-MOR-FA NPs for improving different rheological and antioxidant properties of yoghurt.

1- Introduction

There has been a notable surge in interest regarding the application of nanotechnology within the food industry, particularly in the creation of packaging products that incorporate certain natural essential compounds aimed at enhancing functional and health attributes (Abd Al-hack et al., 2020) [1].

Among the materials that have shown promising potential in this area are proteins that are characterized by their outstanding carrier characteristics and their ability to form effective nanostructures suitable for active biomolecule delivery systems, such as antioxidants and vitamins [2-5]. Moringa tree is known as drum or hot radish, a durable tree belonging to the Moringaceae family. Also goes by the names "wonders," "life tree," and "diamond plants," and is frequently planted due to its fast growth, tolerance to drought, and nutritional qualities. Studies showed that Moringa (*Moringa Oleifera*) has a wide range of antioxidant phenolic compounds. The antioxidant characteristics of phenolic compounds are important in improving the therapeutic and healthful qualities of food such as fortified dairy products [7].

According to recent research, adding phenolic compounds to dairy improves its resistance to oxidation and lowers the risk of unsaturated fat damage, which increases its shelf life and improves its nutritional content [8-9]. According to a study by Gutiérrez-Dorado et al. [10], the incorporation of plant-based substances into dairy products not only improves their taste and sensory characteristics but also increases the health benefits of using them, such as lowering harmful cholesterol levels (LDL) and improving cardiovascular health. According to Singh et al. (2022) [11], adding up to 0.2% Moringa extract to milk enhanced its antioxidant activity and overall phenolic content while retaining an acceptable sensory evaluation. The growth of pathogenic bacteria like *Staphylococcus aureus* and *E. coli* is inhibited by milk fortified with Moringa, according to Kumar *et al.* (2020) [12]. Besides that, they found that adding Moringa extract extends the product's shelf life.

The objectives of this study were to prepare a WPI nanoformulation loaded with Moringa leaf extract and surface-functionalized with FA, and then to study its effect on the chemical,

rheological, and sensory properties of yogurt. These NPs were added to skim milk, and yogurt was produced from the mixture. The effects of this addition on the chemical, physical, and sensory properties of yogurt were studied under different conditions.

2-Materials and methods

1.1. Materials

Skim milk powder was obtained from the French company Regilait, WPI > 90 % protein was supplied by Flso com. (Turkey). Moringa leaves were taken from mature *Moringa oleifera* trees, leaf samples were collected, washed, dried in an oven under vacuum, ground, sieved, and the fat was removed from the powder using solvent extraction methods. FA (99.0 % purity), NaOH, citric acid, H₂SO₄, HCL, and K₂SO₄ from Sigma Aldrich (USA), and yogurt starter was obtained from Danisco.

1.2. Methods

1.2.1. Preparation of WPI-MOR-FA nanocomposite

To prepare the WPI-MOR-FA nanocomposites, 10 g of whey protein isolate (WPI) were carefully dissolved in 100 mL of deionized water. The pH of the mixture was adjusted to 8 using 0.1 M NaOH, and a small amount of sodium azide (0.02% w/v) was added to keep the solution free from microbial growth. The mixture was stored overnight in the refrigerator to stabilize. To remove any trapped air, the solution was degassed under vacuum for 30 minutes, then gently heated at 80 °C for 20 minutes, a step that helped unfold the whey proteins. Afterwards, the solution was diluted with deionized water to reach a final protein concentration of 2% (w/w). At this stage, moringa leaf powder (2 mg/mL) was added and mixed for 30 minutes with a magnetic stirrer, while the pH was adjusted back to 7. To improve homogeneity, the solution was sonicated for 30 seconds, and finally, the mixture was freeze-dried, resulting in the desired nanocomposite powder. Prepared nanocomposite (10% in water) was mixed with equal volume of 10% of both FA for 24 hours at 4 °C. NaOH was used to adjust the pH of the prepared mixed to

7.0, then citric acid (47.0%) was used to lower the pH to 3.0, as a precipitate of WPI- MOR-FA was formed quickly, and the precipitate was allowed to settle for 24 h at a 4 °C and precipitate was collected using centrifugation and freeze dried [14].

1.2.2. Characterization of nanoparticles of WPI- MOR-FA nanocomposite

1.2.2.1. Size, distribution and zeta-potential

The size, dispersion and zeta potential of the prepared nanoparticles were estimated using the Zetasizer Nano-ZS instrument (Malvern instruments: UK). Ultrasound was applied with 15 seconds in an ice-cold water bath having prepared them in a solution containing a concentration of 1 mg/ml in distilled water - nanostructure measurement -three replicates were made at room temperature.

1.2.2.2. Scanning electron microscopy (SEM)

The study of the nanocomposite was done on an SEM (Oberkochen, Zeiss, LEO 1525, Germany). This dry nanocomposite was put on aluminum supports, having conductive carbon tapes on both surfaces, and was coated with a 50/50 blend of Au/Pd. The scans used accelerating voltage of 50kV.

1.2.2.3. Transmission electron microscopy (TEM)

Morphology and structure of WPI-MOR-FA were studied using a TEM (Carl Zeiss AG - Zeiss EM900, Germany) at an acceleration voltage of 40 kV. Fifteen microliters of nanoparticle suspension (100 µg/ml, subjected to 20 seconds of sonication) were applied to a 400-mesh formvar-coated copper TEM grid. The grid was rinsed twice with distilled water and then air-dried in a vacuum desiccator.

1.2.2.4. Study of the effect of pH on the stability of nanocomposite particles

The stability of the nanoparticles at pH 7.4 and 2 was calculated according to Wang *et al.*, 2022 [15].

1.2.3. Estimation of the antioxidant activity of WPI-MOR-FA nanocomposite

Twenty grams of dry weight of each sample was weighed and added to 20 mL of ethanol and incubated for 2 h at 4 °C. This mixture was filtered using Whatman No. 1 filter paper to remove any remaining powder. The filtered mixture was then centrifuged at 2500 rpm for 10 minutes, resulting filtrate was used to measure antioxidant activity following the method described by Thanigaivel *et al.*,2014 [16] using ascorbic acid as a control.

2.2.4. Yogurt manufacturing

Skim milk reconstituted to 10% in distilled water was used in making yogurt. The mixture was separated into four groups (control (without additive) and T1, T2 and T3), which were added with 0.1, 0.2 and 0.3 % of WPI-MOR-FA nanocomposite, respectively. All the mixtures were heated to 90 degC and left to cool to 43 degC and inoculated with 0.02% starter culture that consisted of *Streptococcus thermophilus* and *Lactobacillus delbrueckii sub sp. bulgaricus*. Inoculated samples were placed into 50 mL of plastic containers and incubated at 42 degC until coagulation took place (342 hours). Then the yogurt was kept at 5 °C during 21 days to be examined further.

2.2.4.1. Chemical and rheological tests for yogurt:

The percentage of moisture, ash, and total acidity, total nitrogen, carbohydrates, fat and acidity were determined according to AOAC,2010 [17], viscosity, spontaneous whey separation, water holding capacity and hardness were estimated according to the method mentioned by Ali & Al-Saadi [17]. The apparent viscosity of yogurt samples was estimated at a 5°C using Brookfield DVII + Viscometer . Spontaneous whey separation was estimated in samples after placed at an angle of 45° at room temperature for a period 10-15 minutes. The resulting liquid was removed by syringe from the surface of the sample, and the extraction process took less than 10 seconds to avoid leakage of the extract from the gel. WHC was estimated by weighing 10 g of yogurt sample, and was exposed to centrifugation at 3000 rpm for 60 minutes. The filtrate was removed and the weight of the residual precipitate gel was taken. WHC was estimated using following equation:

$$\text{WHC}\% = [1 - (w_2 / w_1)] \times 100$$

W1 / sample weight (10g), W2 / fluid weight. .

A Texture Analyzer (TA) was used to determine yogurt hardness at 20 ± 2 °C. The parameters used were: cylindrical probe; the velocity of penetration 1.0 mm /s; the distance of penetration 10 mm with 5.0g trigger. Measurement of the strength of gels was done in triplicates [18]. Sensory analysis of yogurt samples was performed in the Department of Dairy Science and Technology - College of Food Sciences / Al-Qasim Green University but on a sensory evaluation form which included taste, flavor, texture, color and appearance.

2.2.5. statistical analysis

One-way ANOVA then followed by the multiple comparisons test of Tukey to compare groups and unpaired Student t-test to compare pairs were the statistical tests used in analyzing data. The definitions of statistical significance were $p < 0.05$, $p < 0.01$ and $p < 0.001$.

3-RESULTS AND DISCUSSION

3.1. WPI- MOR-FA nanocomposite characterization

3.1.1. Estimation of the zeta potential of the prepared nanocomposite

Nano-ZS Zetasizer (Malvern Instruments Ltd, UK) measurements of zeta potential of the prepared nanocomposite gave a -32.0 ± 10.9 mV, good particle stability and low aggregation

tendencies (Fig. 1). This shows the efficiency of the process of loading the bioactive compound through the repulsion of the charges available on the surface of the nanoparticle and the charges in the middle. The dispersion solution is immersed in it and then stabilizes, in which the value of the zeta potential must exceed $7.36 \pm$ mV, which indicates the extent of the stability of the manufactured nanoparticles and their lack of agglomeration, or gathering together due to electrical forces at a temperature of 30 °C. A dispersion coefficient of 0.030 was obtained. ~ millivolts, which is an indicator of sample heterogeneity, which should not exceed 0.2 for most particles that are within nanotechnology specifications, and this is what the previous study showed (Shnoudeh & Alkelany,2019) [19].

As shown in Fig. 2 (a), the size of the nanoparticle of the WPI alone was determined to be 120.8 ± 56.04 nm. The nanoparticle size of the WPI loaded with the MOR extract was determined to be 175.1 nm, Fig. 2 (b). The nanocomposite size of the WPI loaded with the MOR extract and coated with FA was determined to be 199.5 nm (Fig. 2 (c)) that it at the highest density concentration of the synthesized particles, and that these results are consistent with the standard dimensions for manufacturing highly effective nanoparticles that meet the bioavailability specifications of the active compound (Fernández et al., 2016) [20] and also agrees with (Giroux & Britten,2010) [21] who indicated that the size of nanoparticles manufactured from whey protein ranges from 100-300 nm.

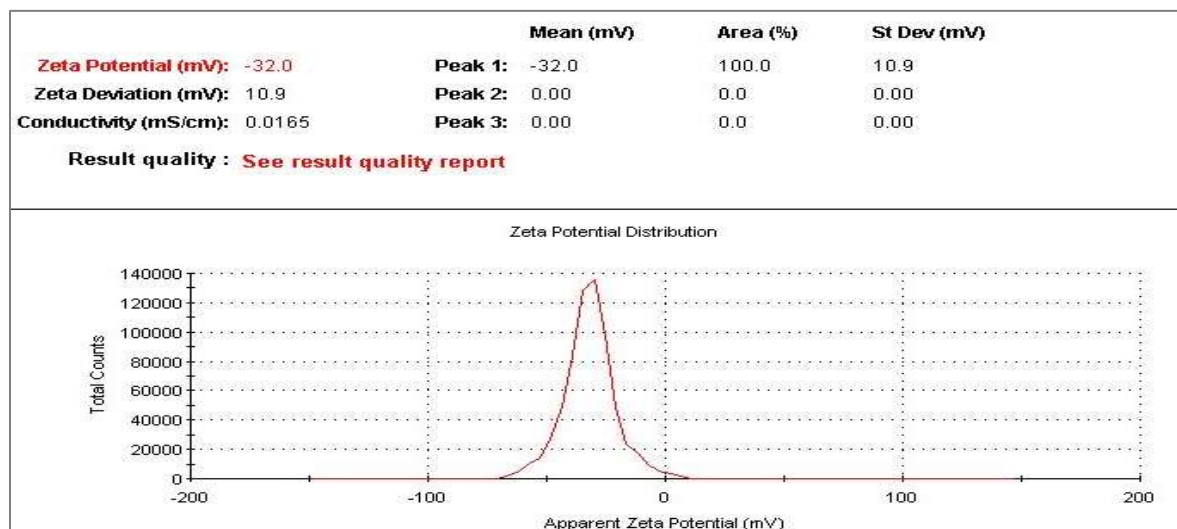


Fig. 1. Zeta potential of the WHEY-MOR-FA nanoparticle

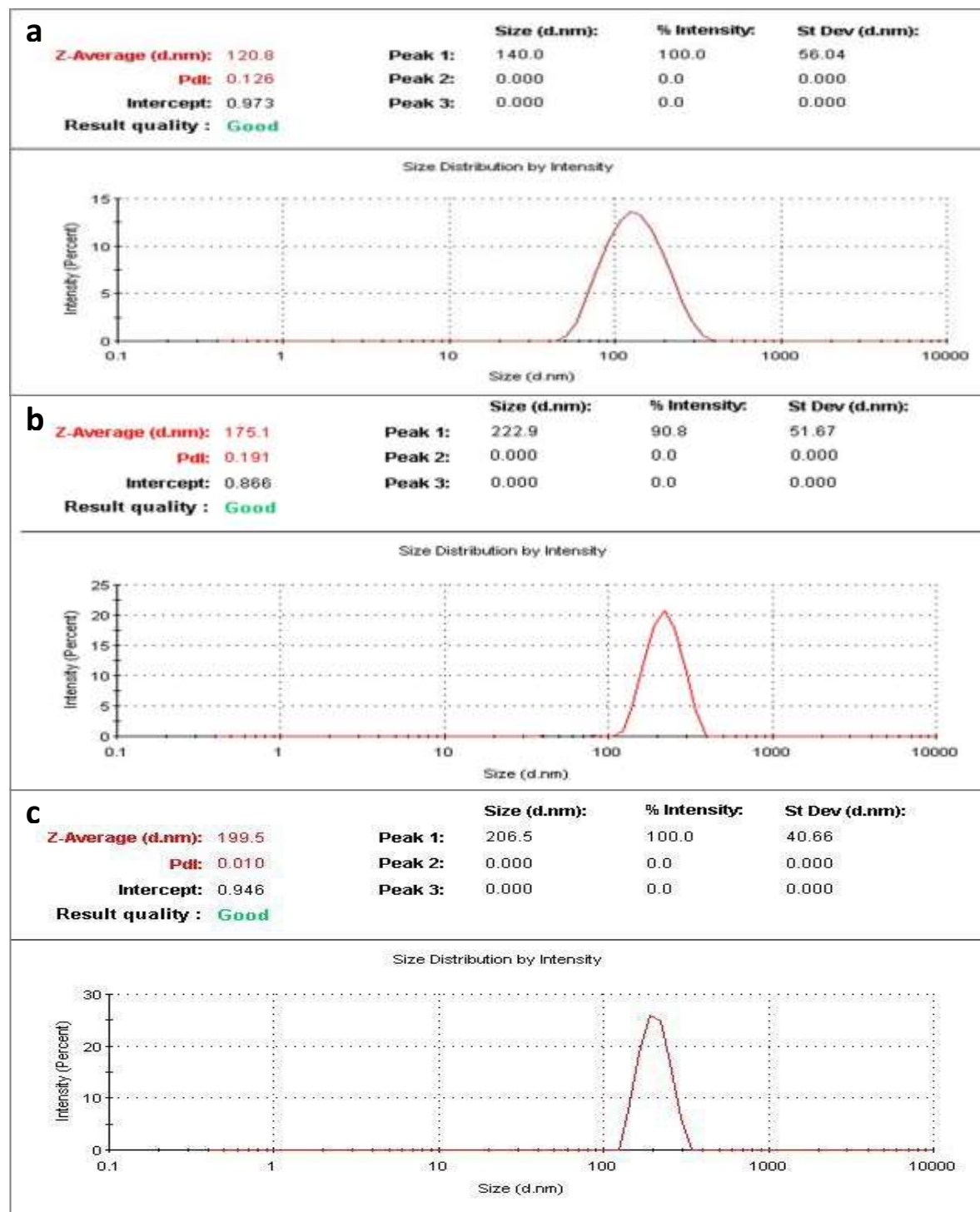


Fig. 2. The size and polydispersity of the WHEY (a), WHEY-MOR (b) and WHEY-MOR-FA (c) nanoparticles powder.

3.1.2. Scanning electron microscope (SEM).

SEM observations (Fig. 3a) indicated that the nanoparticles formed by the whey protein was mostly of a spherical

morphology with a homogenous size distribution. This shows that the creation of the nanoparticles as well as the following freeze-drying process was realized

successfully. In the case of loading these particles with Moringa leaf extract (Fig. 3. (b)) there was no apparent difference in the shape of the spheres, only the surface became rougher. This could be explained by the fact that the incorporation of the phenolic and biologically active compounds contained in the extract into the particle surface indicates the success of the loading process. In the case of the particles covered with FA (Fig. 3. (c)), a more unitary form of the sphere and a small rise of the size were observed, which means that the coating formed an outer layer on the particle surface that improved its structural stability in aqueous media. The findings are

in line with the findings of (Patel et al.,2019) [22], who reported that protein particles take the shape of a sphere when prepared by freeze-drying and that the loading of plant extracts causes a change in the surface but does not significantly alter the shape of the particle. Other related studies, including (Sadeghi et al.,2020) [23], study, have demonstrated that the coating of nanoparticles with bioactive compounds like FA increases the shape regularity and the colloidal stability of the nanoparticles, which is consistent with the results presented in this work.

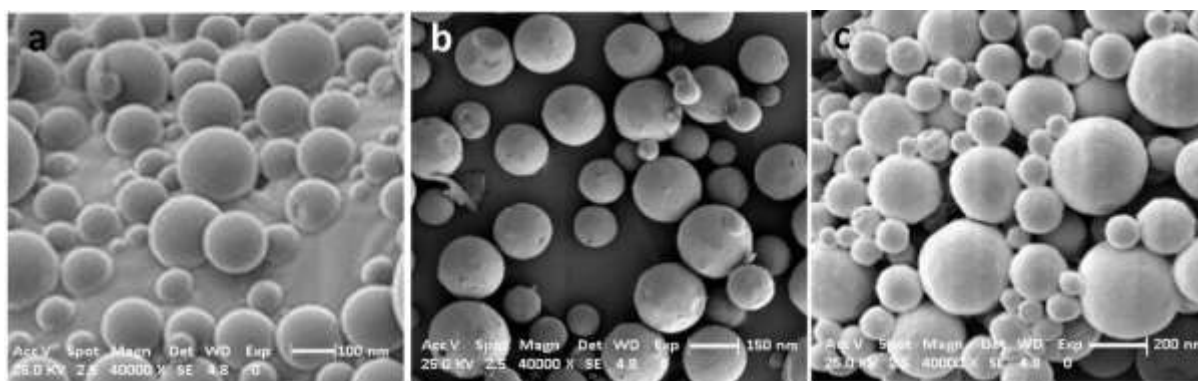


Fig. 3. Scanning electron microscope (SEM) image of WPI nanoparticles (a), WPI nanoparticles loaded with Moringa leaf extract powder (b), and nanocomposite particles formed from WPI loaded with Moringa leaf extract powder and coated with FA (c), respectively.

3.1.3. Transmission electron microscopy (TEM)

The TEM picture of the nanocomposite formed with the help of whey proteins loaded with Moringa leaf extract and covered with FA indicated that the obtained particles were semi-spherical and the nanoscale dimensions were relatively regular (Fig. 4). The image shows that the particles were between 50 and 200 nm in diameter (with some small agglomerations, probably caused by drying of the samples or by the effects of the protein and the active components of the plant extract). This outcome is an indication of the effectiveness of the loading/encapsulation procedure and a demonstration that the

freeze-drying procedure is effective in preserving the spherical geometry of the particles and not deforming them. Visible damage or cracks. The given nanostructure can be utilized in the food and pharmaceutical industries, as an alternative to the regular, spherical structure, the given nanostructure promotes biodistribution and elevated stability (Zhao et al., 2021) [24]. As was confirmed in (Saini et al.,2016) [25], the active phenolic compounds and bioflavonoids present in Moringa leaf extract are able to form stable complexes when loaded onto nanocarrier particles like whey proteins, thereby increasing their antioxidant activity, and their efficacy in biodelivery.

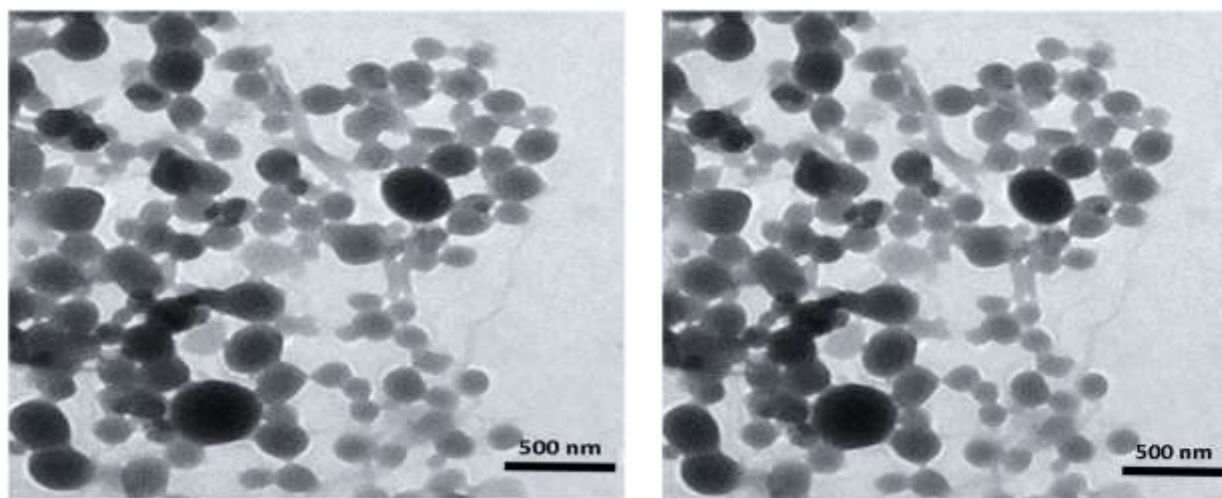


Fig. 4. TEM image showing the internal morphology of a nanocomposite particle prepared from WPI loaded with Moringa leaf extract powder and coated with FA at 120 kV and 40,000 × magnification.

3.1.4. Effect of pH on stability of Moringa leaf extract nanocomposite

pH is one of the important factors affecting the stability and decomposition of protein nanoparticles, as when the pH values differ, the surface charge of the particles changes, which affects the repulsion or electrical attraction between particles, and therefore affects their structural stability. The stability of nanoparticles was studied in solutions with pH 2 and 7.4 under laboratory conditions similar to those of the human digestive system, and it is clear from Fig. 5 that the decomposition rate of the nanoparticle varies significantly with the pH value. At pH = 7.4 the decomposition was much higher compared to the pH = 2, so after 120 minutes the decomposition rate at pH = 7.4 reached about 85%, while it did not exceed 25% at pH = 2, and this result can be explained by the

fact that the nanoparticles depend in their structure on the proteins of the whey, which consist mainly of β -lactoglobulin and α -lactalbumin, which have an acidic pI < therefore at pH 2 it contains few charges remain with nanoparticles and does not dissolve, while at pH 7.4 these proteins become more soluble and their release from nanoparticles increased (Wang *et al.* 2022) [14]. Previous studies have confirmed that whey proteins are a suitable material for forming nanoparticle systems that exhibit pH-dependent behaviour, decomposing slowly in acidic media and decomposing faster in neutral and basic media (Sahu *et al.*, 2020) [26]. This is also consistent with (Onsekizogiu & Gunasekaran, 2017) [27], who stated that the level of release of the whey proteins from nanoparticles at low pH levels was 28% and at neutral pH, it was even higher (95%).

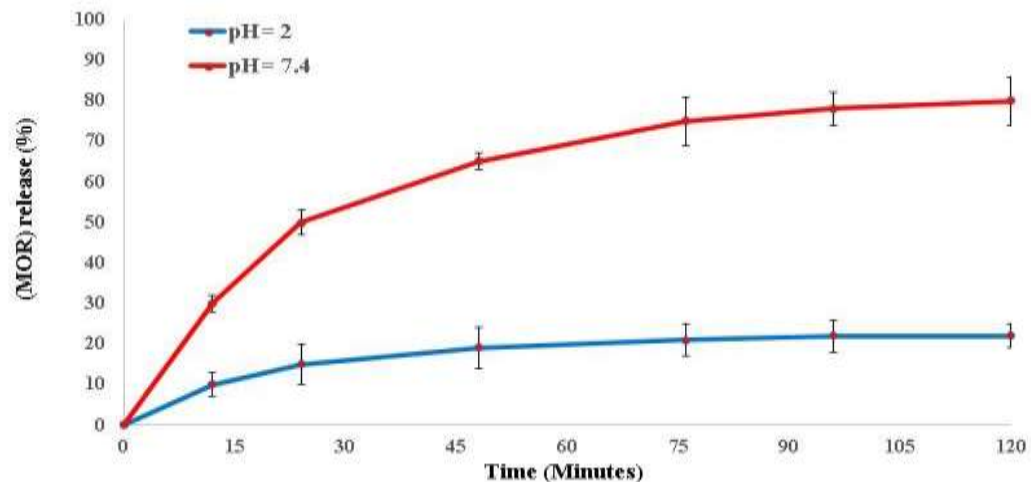


Fig. 5. Effect of pH and time on stability of nanoparticles of the nanocomposite

3.1.5. Antioxidant efficacy of nanocomposites:

The results in Fig. 6 showed that loading Moringa extract in whey protein nanoparticles and FA led to a significant improvement in antioxidant activity as measured using the ABTS and DPPH tests. Control (ascorbic acid) inhibitory activity was averaged at approximately 20% on the ABTS and DPPH tests, which confirmed weak antioxidant behavior, and the antioxidant activity of nanoparticles formed with only whey proteins was approximately 25% on the ABTS test and 30% on the DPPH test, which confirmed that the antioxidant behavior of whey proteins was weak. Nanoparticles made of whey proteins that

were loaded with Moringa extract and FA had the greatest antioxidant activity, and the rate at which free radicals are inhibited was about 70% in both tests. These findings provide evidence that the antioxidant ability of Moringa leaf extract and FA is high, and their inclusion in whey protein nanoparticles led to their enhanced effectiveness. According to (Kumar et al.,2019) [28], Moringa is a potent antioxidant drug and has been found to react with other free radicals including hydroxyl radicals. These findings in this work are in line with (Li and Zhao 2020) [29], who observed that loading FA into nanoparticles enhanced their capacity to eliminate free radicals, particularly when combined with other antioxidant compounds, including flavonoids

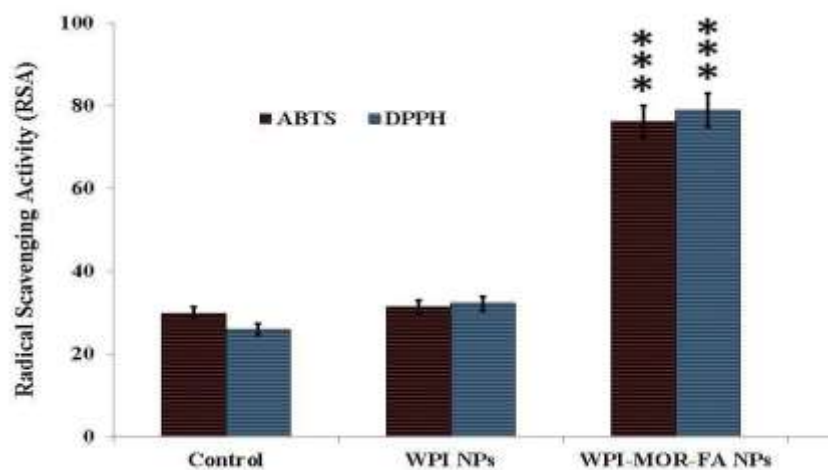


Fig. 6. Radical scavenging activity of control, WPI NPs, and WPI-MOR-FA tested with ABTS and DPPH. The WPI-MOR-FA NPs' radical scavenging activity (RSA) went up a lot. *** mean p value ≤ 0.005 , respectively.

3.2. Chemical tests for yogurt:

3.2.1. Estimating the percentage of fat for different curd treatments:

When estimating the fat percentage in this experiment, as shown in Table (1), it was 0.1 in all treatments. This is because the milk used was skim milk, and the extract and WPI were low-fat. This is consistent with the findings of (Ibrahim, 2016) [۳۰], who showed that yogurt made from skim milk had a fat percentage of 0.1% throughout the 14-day storage period. The statistical analysis findings prove that there are no significant differences ($P < 0.05$) in fat percentage of the various yogurt treatments after the first day and the 21-day storage period, where the LSD value was (0.00 NS).

3.2.2. Estimating the moisture content of different yogurt treatments

Moisture content in the control treatment after one day of storage was 88.5%. This percentage is close to what (Al-Suwudi *et al.*, 2024) [۳۱], found for fat-free yogurt, which was estimated at 88.1%. In treatments T1, T2, and T3, the moisture content after one day of storage were 88.3, 88.1, and 88.0%, respectively. After a 21-day storage period, the moisture contents for treatments C, T1, T2, and T3 changed to 88.0, 88.0, 87.7, and 87.6%, respectively. The results of moisture for all treatments showed a slight decrease with the progress of the storage period and this attributed to the natural evaporation of water during storage. These results are consistent with what (Hashemi *et al.*, 2021) [۳۲], indicated, stating that fermented dairy products, such as yogurt, may exhibit a limited decrease in moisture content during

storage due to slow evaporation processes or physical interactions between proteins and water, particularly in products with low fat content, where water loss is more noticeable compared to full-fat varieties. Moisture content decreased with increasing the added percentage of nanocomposite. This was due to a slight increase in solids resulting from the addition. The results of the statistical analysis indicated no significant differences ($P < 0.05$) in the moisture content between the different yogurt treatments after the first day and during the 21-day storage period, with the LSD value reaching 3.219 NS.

3.2.3. Estimation of protein content in different yogurt treatments:

Protein (%) after one day of storage for the control treatment was 4.71% and for treatments T1, T2, and T3 were 5.05%, 5.14%, and 5.21%, respectively. This increase in protein percentage is attributed to the use of whey proteins and extract powder in preparing the nanocomposite. This is consistent with what was found by Al-Darwash *et al.*, 2014 [33], who indicated that adding plant-based materials to yogurt increases the protein percentage. The results also showed a slight decrease in protein content after a 21-day storage period for C, T1, T2, and T3 to 4.62, 4.96, 5.06, and 5.11%, respectively. However, the percentage remained high for treatments containing additives compared to the control treatment. The results of the statistical analysis indicate non-significant differences ($P < 0.05$) in the protein content between the different yogurt treatments after the first day and during the 21-day storage period, with the LSD value reaching 0.527.

Table (1): Chemical composition of the control and the yogurt treatments containing Moringa nanoparticles

Treatment	Storage time (day)	Moisture %	Protein %	Fat	Carbohydrate%	Ash %
C	1	88.5	4.71	0.1	5.65	0.70
	7	88.3	4.71	0.1	5.68	0.70
	14	88.1	4.67	0.1	5.72	0.70
	21	88.0	4.62	0.1	5.75	0.70
T1 (Yogurt with 0.1% Moringa nanoparticles)	1	88.3	5.05	0.1	5.55	0.71
	7	88.1	5.05	0.1	5.58	0.71
	14	88.0	5.00	0.1	5.60	0.71
	21	88.0	4.96	0.1	5.63	0.71
T2 (Yogurt with 0.2% Moringa nanoparticles)	1	88.1	5.14	0.1	5.45	0.73
	7	88.0	5.14	0.1	5.49	0.73
	14	88.8	5.10	0.1	5.52	0.73
	21	87.7	5.06	0.1	5.55	0.73
T3 (Yogurt with 0.3% Moringa nanoparticles)	1	88.0	5.21	0.1	5.35	0.75
	7	87.7	5.20	0.1	5.38	0.75
	14	87.7	5.15	0.1	5.43	0.75
	21	87.6	5.11	0.1	5.45	0.75
L.S.D.		3.219 NS	0.527 *	0.00 NS	0.902 NS	0.189 NS

3.2.4. The percentage of carbohydrates in different yogurt treatments:

The level of carbohydrates after one day of storage under control treatment (C), treatment T1, T2 and T3 was 5.99 percent, 5.84 percent, and 5.93 percent, respectively. After 21 days, the ratios of carbohydrates became 6.58, 6.23, 6.41, and 6.44% of treatment C, T1, T2 and T3, respectively. The findings revealed that an increment in the percentage of carbohydrates with respect to increasing storage time was noted among the treatments. This rise can be explained by the rise in the proportion of total solids as a result of the minor evaporation that took place in the samples throughout the storage period (Al-Saadi, 2014) [34]. The outcome of the statistical measure shows that the samples of carbohydrate percentage within the varying yogurt treatments do not significantly differ at the level of P,0.05, and in both the case of the first day and the storage duration of 21 days since the L.S.D value was (0.902 NS).

2.2.6. Ash percentage in yogurt treatments.

Table (1) revealed that the percentage ash content in the control group after one day of storage was 0.70% in treatment T1, 0.71, 0.73 and 0.75 in treatments T2, T3 and T4 respectively. The findings reveal that all of the nanocomposites have more ash, as compared to the control. This is because of the structure of the nanocomposite since it has Moringa extract and whey protein which have substances which help in elevating the ash content. These findings are in agreement with those of (Aziznia et al.,2008) [35], who reported higher ash content of low-fat yogurt treatments to which whey proteins were added. The ash content of the treatment C, T1, T2, and T3 after 21 days storage was 0.70, 0.71, 0.73 and 0.75% respectively. The chemical analysis results showed a remarkable stability in the ash content of all yogurt samples throughout the 21-day storage period, and this attributed to the fact

that ash represents the total content of inorganic minerals, which are relatively stable components and are not affected by the minor biochemical or microbial changes that may occur during storage [36]. The statistical analysis findings prove that there are no significant differences ($P < 0.05$) in fat percentage of the various yogurt treatments after the first day and the 21-day storage period, where the LSD value was (0.189 NS).

2.2.7. Total acidity %

The results in Fig. 7 show the total acidity of the yoghurt samples. Acidity in control after one day of storage was 0.80%. This result is very close to what Jasim & Al-Saadi (2020) [37] found, which was 0.81% for fat-free yoghurt. The total acidity in treatments T1, T2, and T3 was 0.76%, 0.74%, and 0.72%, respectively. The results indicate a decrease in the total acidity of Moringa nanocomposite treatments due to the nanocomposite's ability to modify the acidity properties. (Zhang *et al.*, (2021) [38], stated that nanocomposites containing proteins and substances that

enhance antioxidants affect the acidity properties of food products. Fig. 7 also showed that total acidity results after 21 days for treatments C, T1, T2 and T3 were 0.95, 0.85, 0.84 and 0.83%, respectively. The findings reflect that there is an upward trend in the total acidity ratio as the storage period increases in all the treatments. The findings are also consistent with the results reported by Kaur and Riar (2020) [39] who have observed that the acidity ratio of the yogurt treatments increases during refrigerated storage due to the presence of the starter and the presence of lactose and its transformation into different organic acid products with lactic acid being the most predominant one. Fig. 7 results revealed that this rise in the acidity ratio was reducing with the rise in the concentration of Moringa nanocomposite added as treatments. The findings of the statistical test reflect that there is significant variation at the level of $P = 0.05$ in the total acidity ratio between the various yogurt treatments at the end of the first day and 21 days of storage where the L.S.D = 0.166*.

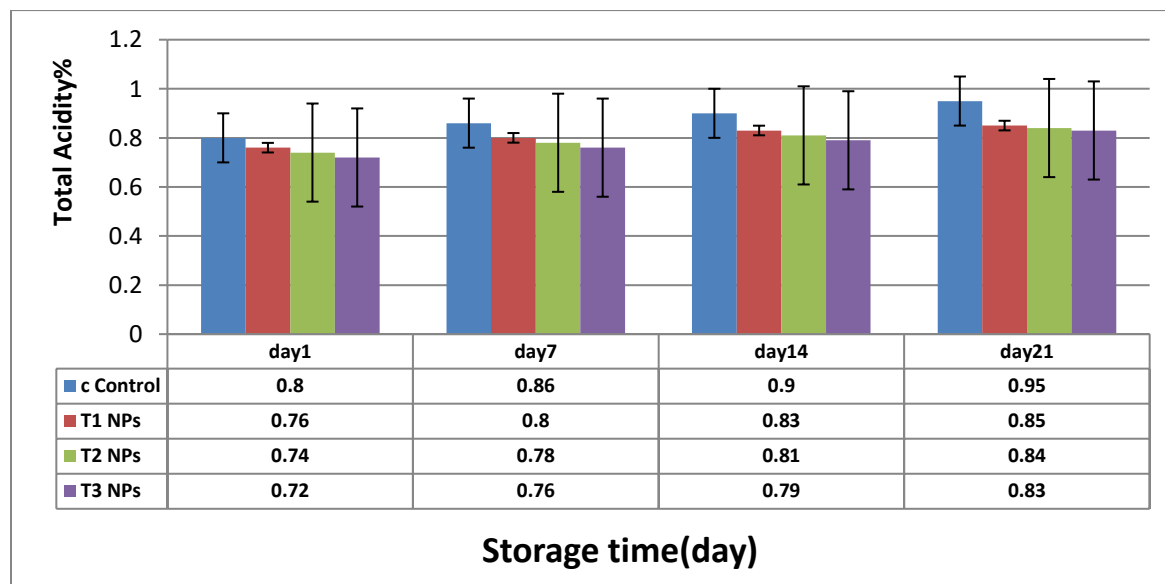


Fig. 7. Total acidity values (%) for yogurt treatments to which nanoparticles was added during storage for 21 days at refrigeration temperature, LSD=0.166 *

2.2.8. Spontaneous Whey Separation

Fig. 8. shows the amount of whey released in different yogurt treatments. The amount of

whey separated from control after 1 day of storage was 1.89 ml/50 ml and for treatments T1, T2, and T3, were 1.07, 0.91, and 0.75 ml/50 ml respectively, and these values changed after 21 days of storage to 3.19, 2.00, 1.68, and 1.40 ml/50 ml for C, T1, T2, and T3 respectively. Results showed that whey leakage gradually increased in all treatments as the storage period progressed from day 1 to day 21, with the highest values recorded at the end of the storage period. The results showed that the control recorded the highest whey separation, while the T3 treatment, to which the nanocomposite was added at a concentration of 0.3%, recorded the lowest rate over the same period. This demonstrates the effectiveness of the nanocomposite loaded with Moringa leaf extract in reducing water loss (whey) in yogurt. This decrease in spontaneous whey separation in treatments containing Moringa leaf nanocomposite is attributed to their high content of natural fibers and proteins, which contribute to

enhancing the water-holding capacity within the protein network structure of yogurt, thus reducing whey separation during storage. This is supported by the findings of (Tang et al.,2017) [40], who demonstrated that the addition of plant extracts or nanocomposites rich in active ingredients may improve the rheological and physical properties of dairy products by enhancing the protein network and its moisture-holding capacity. It was also observed that the decrease in whey leaching was more pronounced with increasing additive concentration. Treatments T1, T2, and T3 showed a clear downward trend in leaching, confirming the direct relationship between nanocomposite concentration and its ability to improve yogurt stability. Statistical analysis results indicate significant differences ($P < 0.05$) in the spontaneous whey leaching rate between the different yogurt treatments after the first day and during the storage period (21 days), with the LSD value reaching (1.075*).

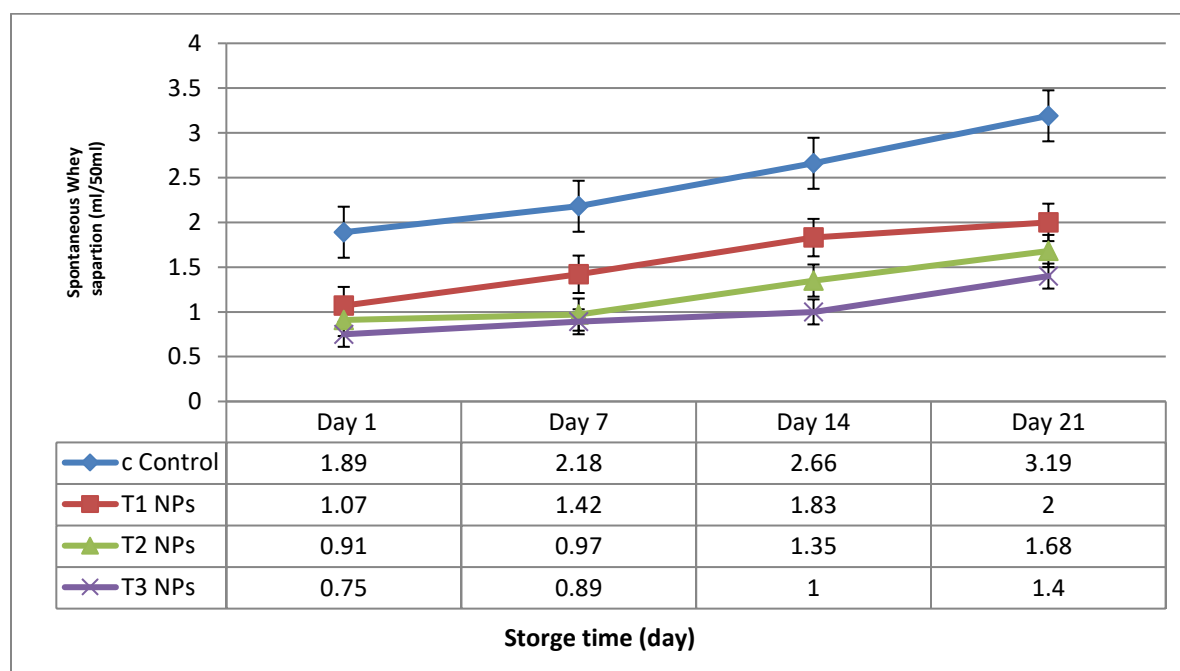


Fig. 8. Spontaneous whey separation values for yogurt treatments to which nanoparticles were added during storage for 21 days at refrigeration temperature. LSD=1.075 *

2.2.9. Water holding capacity :

Water holding capacity of yogurt varies significantly depending on the type of additive, its concentration, and storage time Fig. 9. In the control treatment, water holding capacity started at 75% after 1 day of storage and then gradually decreased over time to reach 59.8% after 21 days. This decrease reflects natural changes in the protein structure of the yogurt as a result of decomposition processes or fluid loss. Water holding capacity for T1, T2, and T3 on day 1 was 80.5, 80.6, and 81.2%, respectively, and these values changed to 66.0, 66.2, and 66.5%, respectively, after 21 days of storage. From the above results, it was clear that treatments T1, T2, and T3 continued to

achieve higher water holding capacity values compared to the control treatment, and this indicates the role of moringa nanoparticles in strengthening the yogurt protein network. These results are consistent with what Zhang *et al.* (2023) [41], indicated, which is that the water-holding capacity decreases during the 21-day refrigerated storage period due to the increase in acidity, which causes the loss of negative charges in milk proteins and increases hydrophobic interactions, reducing the protein network's ability to retain water. Statistical analysis results indicate significant differences ($P < 0.05$) in the water-holding capacity between the different yogurt treatments after the first day and during the 21-day storage period, with the LSD value reaching 7.16*.

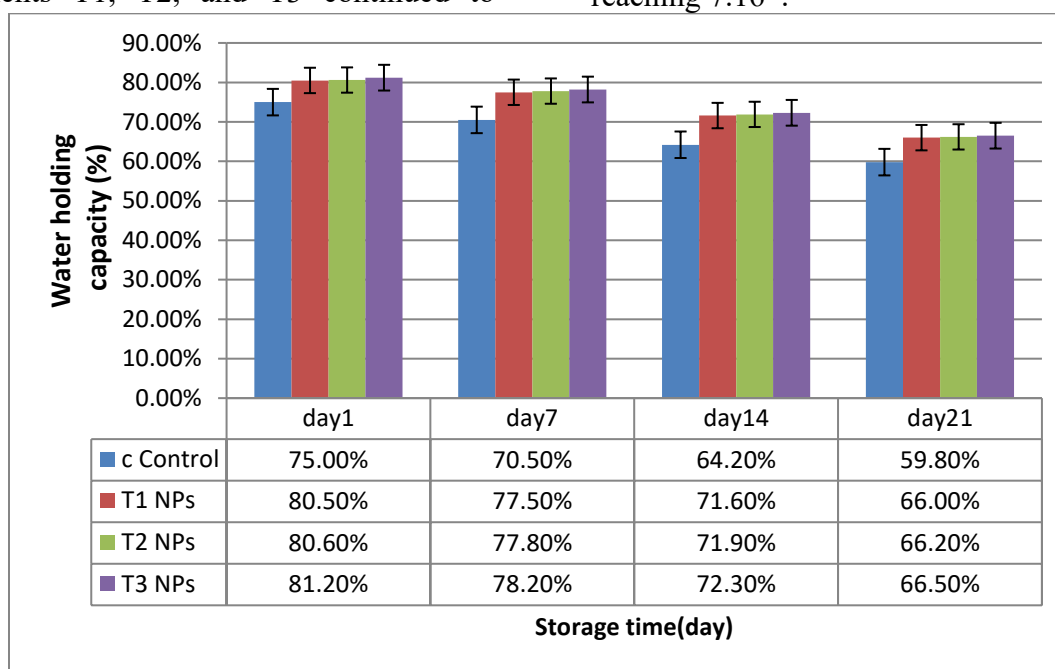


Fig. 9. Water holding capacity values of yogurt treatments to which nanoparticles added during storage at refrigeration temperature. LSD=7.16 *

3.2.9. Viscosity:

In this study, it was observed that samples treated with nanocomposites achieved higher viscosity values compared to the control treatment Fig. 10., especially at the higher concentration (0.3%). This increase is attributed to the ability of these additives to modify yogurt structure. In control, viscosity

was lower compared to the other samples at all time periods. After 1 day of storage, the viscosity reached approximately 1380 centipoise, while the viscosities for T1, T2, and T3 were 1462, 1455, and 1459 cp, respectively. This increase can be explained, according to Alam & Islam, 2018 [42], by the fact that Moringa nanoparticles contain

proteins and carbohydrates that increase viscosity, beside that Kumar& Singh (2019), found that nanocomposites interact with the substances present in yogurt, leading to the formation of three-dimensional networks and changes in molecular structure, thus increasing viscosity. After 21 days of storage, the viscosities of C, T1, T2, and T3 changed to 1366, 1442, 1440 and 1446 cp, respectively. Over time, a slight decrease in viscosity was observed in all samples, including the control treatment. This decrease can be explained, according to Saha

& Das 2020) [43], by the fact that yogurt undergoes decomposition or chemical reactions over time, leading to changes in its composition and properties, including viscosity. However, samples containing the nanocomposite remained higher in viscosity compared to the control treatment even after 21 days. The statistical analysis results reveal that there is a significant difference ($P<0.05$) in the values of viscosity in the various yogurt treatments after the first day and after the 21 days of storage. where the value of L.S.D was 74.98*.

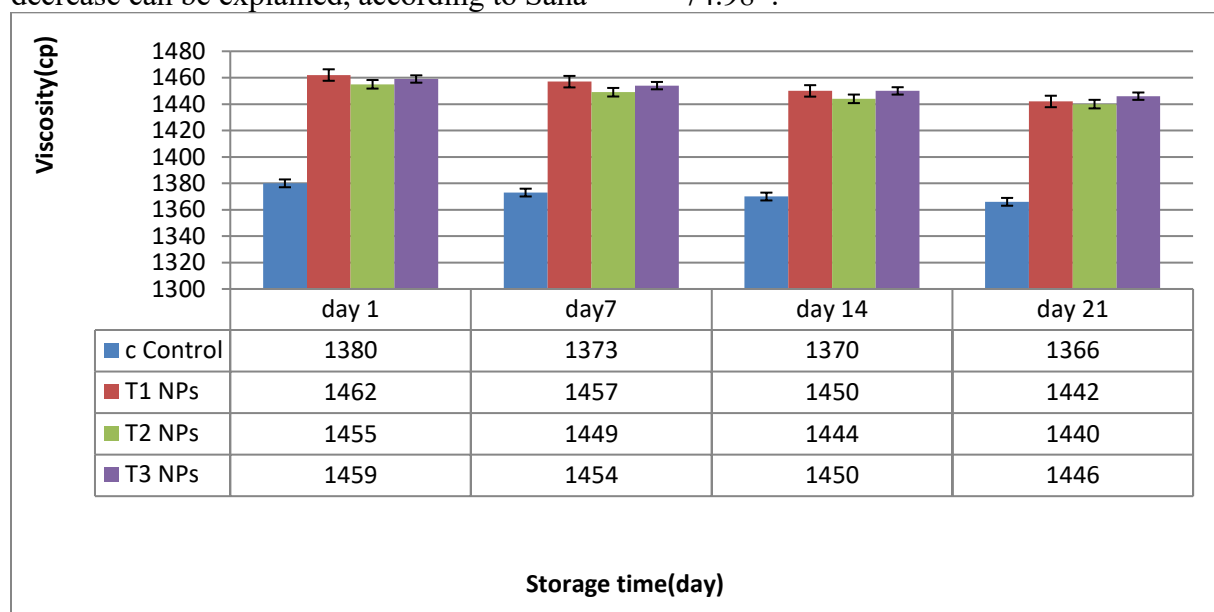


Fig. 10. Viscosity values for yogurt treatments containing moringa nanoparticles during storage for 21 days at refrigeration temperature. LSD=74.98*

3.2.10. Hardness

Fig. 11. showed hardness data for yogurt samples during storage for 21 days. In the control treatment, hardness was the lowest compared to the other samples at all time periods. After 1 day, the hardness was 77.7 g, while it increased slightly after 21 days to reach 78.7 g. Hardness for treatments H1, H2, and H3 after one day were 99.8, 102.9, and 106.1 g, respectively, and these values changed after 21 days to 101.5, 104.9, and 108.0 g, respectively. This indicates that natural yogurt has a less rigid gel structure compared to samples containing additives. This is attributed to the effect of active

ingredients such as polyphenols and proteins on strengthening the gel network produced by milk proteins [44]. Nanoparticles play a significant role in enhancing hardness due to their chemical and physical properties, which contribute to improving gel structure due to the ability of nanocomposites to enhance the bonding between protein molecules [45]. Addition of Moringa nanocomposites effectively contributes to enhancing the hardness of yogurt, a finding supported by (Darwish *et al.*, 2021) [46], who indicated the ability of plant extracts and nanocomposites to improve texture properties and meet consumer requirements in terms of an

acceptable consistency and smooth texture. The results of the statistical analysis indicate the presence of significant differences at the level of $P < 0.05$ in the hardness values

between the different yogurt treatments after the first day and during the storage period of 21 days, where the L.S.D value reached 9.61*).

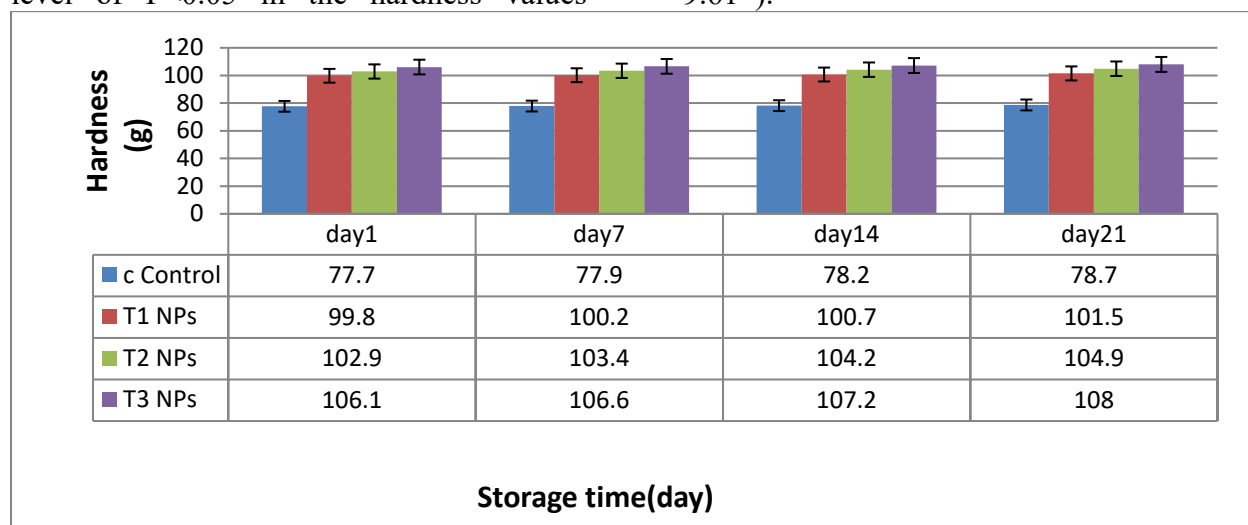


Fig. 11. Hardness values for yogurt treatments to which nanoparticles were added during storage for 21 days at refrigeration temperature. *($P < 0.05$) $LSD = 9.16^*$

3.2.11. Antioxidant activity of yogurt

Fig. 12. shows antioxidant activity for different experimental yogurt treatments. Antioxidant activity for C, T1, T2, and T3, after 1 day of storage, were 85.686, 89.717, 90.067, and 90.313%, respectively and these values changed after 21 days of storage to 83.785, 88.77, 88.401, and 89.489%, respectively. The results showed that all treatments enriched with Moringa nanoparticles recorded higher radical scavenging rates compared to the control sample during all storage periods, indicating increased antioxidant activity in these samples. The T3 treatment with 0.3 percent Moringa leaf extract showed the highest value of radical scavenging activity. This antioxidant activity increase is because of the existence of polyphenolic compounds and flavonoids in Moringa leaves that is known to have a good free radical scavenging potential (Anwar et al., 2007) [47]. It is also comparative with the findings of Jambi

(2018) [48] which showed that the rate of free radical scavenging increases with the percentage of materials with high concentrations of phenolic compounds. All samples showed a gradual reduction in radical scavenging activity with increasing storage period due to degradation of certain active compounds and due to exposure to storage factors of light, temperature and chemical reactions. This reduction was greater in control than in treatments T1, T2, and T3 since coating of these compounds in a FA-coated nanocomposite increased their bio-stability and prevented their degradation during storage (Sharma and Arora, 2021) [49]. The results of the statistical analysis indicate the presence of significant differences at the level of $P < 0.05$ in the free radical inhibition rates within 15 minutes between the different yogurt treatments after the first day and during the storage period of 21 days), as the L.S.D value reached (4.437*).

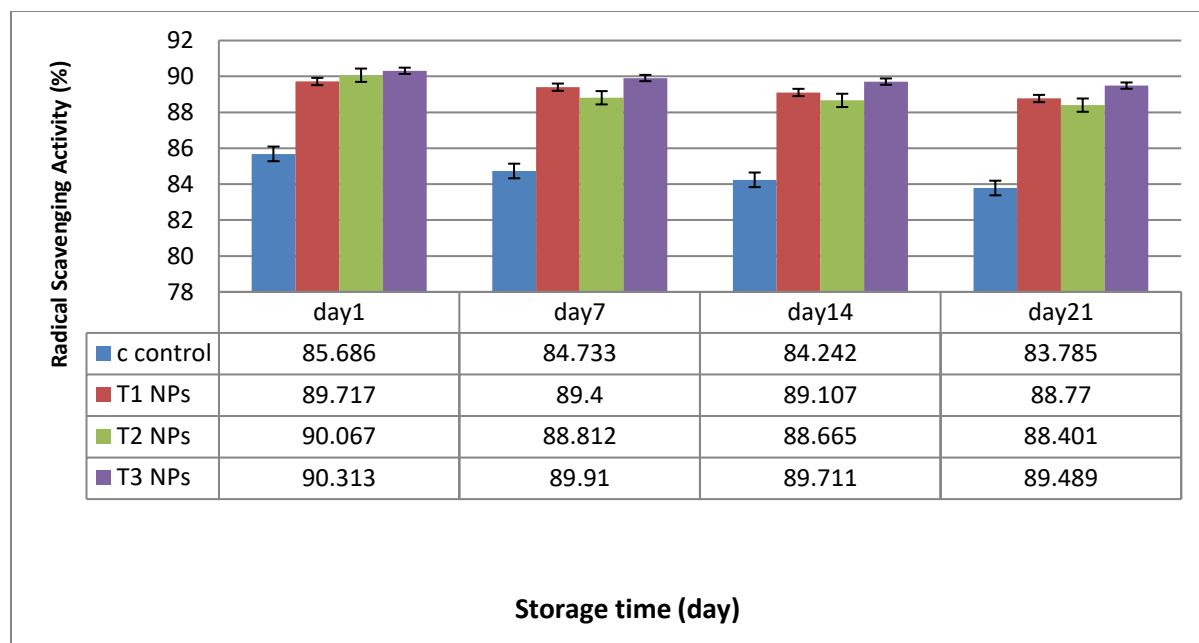


Fig. 12. shows the values of DPPH free radical suppression for yogurt treatments to which nanoparticles were added during storage for 21 days at refrigeration temperature.

2.3. Sensory evaluation of different yoghurt treatments

Table 2 shows of sensory evaluation score of yogurt under different treatments and during storage for 21 days.

The results in Table 2 showed that the total score for C, T1, T2, and T3 after 1 day of storage were 89, 95, 96, and 95, respectively, and during storage, these values changed until they reached 80, 91, 92, and 89, respectively, after 21 days of storage. All yogurt samples treated with the addition of nanoparticles had higher scores than control treatments throughout the storage period. This means that the additives have a positive impact on the flavor, flavor, texture, consistency, acidity and appearance than the

control treatment. The presence of WPI made all the nano-compound treatments whiter and tastier than the other treatments. This is in line with most of the studies that have been conducted on the effectiveness of using nano-systems in enhancing the quality of dairy products. According to a study by (Bhushani & Kumar 2017) [50], addition of nano-compounds to fermented milk did not have any adverse impact on the antioxidant properties of fermented milk, but improved the antioxidant properties of fermented milk. The results of the statistical analysis indicate that the sensory evaluation scores differ significantly at $P < 0.05$ level of the various yogurt treatments after the first day and during the storage period (21days) of yogurt where, the level of L.S.D was (4.611 *).

Table (2): Sensory evaluation results for yogurt fortified with moringa nanoparticles during storage at $5 \pm 1^\circ\text{C}$ for 21 days.

Treatment	Storage time (day)	Taste& flavor (45)	Texture (30)	Appearance (10)	Acidity (10)	Package (5)	Total (100)
C	1	41	26	10	7	5	89
	7	39	25	9	7	5	85
	14	38	25	8	7	5	83

	21	37	24	8	6	5	80
T1	1	24	29	10	9	5	95
(Yogurt with 0.1% Moringa nanoparticles)	7	24	28	10	9	5	94
	14	14	28	10	9	5	93
	21	41	27	9	8	5	91
T2	1	34	29	10	9	5	96
(Yogurt with 0.2% Moringa nanoparticles)	7	43	82	10	9	5	95
	14	24	28	10	9	5	94
	21	42	28	9	8	5	92
T3	1	24	29	10	9	5	95
(Yogurt with 0.3% Moringa nanoparticles)	7	24	28	10	9	5	94
	14	42	27	10	9	5	92
	21	41	26	9	9	5	89
L.S.D.		2.445 *	2.837 *	1.453 *	1.258 *	0.00 NS	4.611 *

.(P≤0.05) *

4-Conclusion

The Moringa leaf extract powder nanocomposite prepared with WPI and coated with FA was a useful compound and enhanced the physicochemical, rheological, biological, and sensory of yogurt. The method employed in preparation induced the formation of nanoparticles which assume a regular spherical form and whose structure is cohesive. The success of the loading process was clearly illustrated by the surface and structural changes observed, increasing the potential of this nanocomposite in the field of functional foods. The developed nanocomposite showed stability regarding good resistance to unfavorable conditions at low pH, the equivalent of the human digestive system. The measurements of zeta potential and dispersion coefficient proved that the size of the coated particles was 199.5 nm, which is regarded as the range of nanoparticles. It was found that the samples enriched with the additives extended the shelf life of yogurt, improved its rheological and antioxidant properties. Sensory evaluation results indicated that consumers accepted the yogurt product to which the nanocomposite was added at all concentrations, as it tasted better and was whiter.

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Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest

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