**Egyptian Journal of Chemistry**

**http://ejchem.journals.ekb.eg/**

# Preparing and Evaluating a Caseinate-Based M. Oleifera Seed oil Nanoemulsion to be Incorporated into Functional Ice Cream

**Amal Abd-alla Mattar[1], Souad Mahmoud El-Desouky[1], Ahmed Abd El-Aziz Elrefaey[2], Wafaa Mahmoud Abd El-Raouf[1], Tarek Nour Soliman[3*]**

[1] Food Science and Technology Dep. Home Economic Faculty, Al-Azhar University, Tanta, Egypt.
[2] Food science and technology department, Mansoura University
[3] *Dairy Department, Food Industries and Nutrition research Institute, National Research Centre, El-Buhouth st, Dokki, Cairo, Egypt.*

**Abstract**

People have constantly looked for bioactive compounds that improve health throughout history. The current study investigates the protection of bioactive components of Moringa oleifera seed oil (MSO) and its use in producing functional ice cream. Four formulations of nanoemulsions were prepared by cold gel techniques using sodium caseinate (NaCas) as an emulsifier and MSO. The nanoemulsions were prepared using 5% NaCas and 5% MSO (T1), 5% NaCas and 10% MSO (T2), 10% NaCas and 5% MSO (T3), and 10% NaCas and 10% MSO (T4). From the results, T1 exhibited the lowest particle size (125.80 nm) and narrow size distribution. In comparison, T4 exhibited particle size (167.50 nm), the lowest PDI value, and the greatest zeta potential, which led to a highly stable emulsion. Specifically, T4 was used to prepare ice cream with 3% MSO. The ice cream samples were divided into control and supplemented with free MSO (T1) or MSO nanoemulsion (T2). Adding MSO nanoemulsion (T2) significantly decreased the pH readings. Also, enhanced the ice cream's total proteins, overrun value, whipping ability, melting resistance, antioxidant capacity, and improved sensory qualities more significantly than the control and free MSO (T1).

Keywords: Moringa oleifera seed oil; Sodium caseinate; Nanoemulsion; Creaming stability; ice cream; ice cream properties.

## 1. Introduction

Ice cream is a consumable product with a market value estimated at 5.75 billion liters, according to the United States Department of Agriculture (USDA) [1]. Furthermore, according to Kilara & Chandan [2]research, ice cream represents 86.7% of the worldwide volume of frozen desserts. Ice cream is a food of significant value, as it contains highly nutritious constituents that benefit human health. These components include milk, an excellent source of proteins, vitamins, and minerals[3]. Ice cream is a frozen dairy product typically consumed as a dessert or snack. It comprises milk and milk products and is often augmented with fruits, flavours, and colours. Ice cream is valued for its significant protein content of high quality and its readily assimilated calcium. Consuming this food item can offer many essential nutrients and serve as a beneficial and pleasurable addition to one's daily caloric intake within a nutritious and well-rounded diet [4]. Ice cream products have recently been fortified with various bioactive ingredients to improve their nutritional properties. Various bioactive components have been incorporated into ice cream, including pomegranate peel phenolic [5], purple rice bran oil [6], whey protein [7]. Contemporary consumers prefer natural foods that are abundant in nutrients and possess potential biological benefits. This trend encourages food manufacturers and researchers to develop novel formulations of ice cream that incorporate various enriching ingredients. Nonetheless, it has been found that commercial ice

* Corresponding author e-mail: tariknour.nrc@gmail.com.; (Tarek Nour Soliman).
Received date 27 September 2023; revised date 10 October 2023; accepted date 04 March 2024
DOI: 10.21608/EJCHEM.2024.239300.8679

cream products do not serve as a rich source of these essential nutrients [8].

Prior studies have suggested incorporating phytochemical-rich oils to augment the nutritional quality of ice cream products. These oils include chia (Salvia hispanica L.) [9], flaxseed oil [10], and hazelnut oil and olive oil [11]. Moringa oleifera seed oil (MSO), which is high content in monounsaturated fatty acids, especially oleic acid, has attracted interest from across the globe due to its superior stability, positive nutritional traits, and therapeutic properties Boukandoul et al.[12]. Commercially called "oil Ben" or "oil Behen." take in the nutritional value, which boasts a remarkable composition of over 70% monounsaturated fatty acids, predominantly oleic acid, and omega-9. It has saturated fatty acids and small amounts of polyunsaturated fatty acids, significantly resisting oxidative degradation [13]. Also, have exceptional benefits, packed with tocopherols, sterols, vitamin E, essential minerals, and bioactive compounds. Phytochemicals display a wide range of biological activities, including antimicrobial properties [14]. anti-inflammatory [15], and provide numerous health benefits, such as anticancer, and anti-cardiovascular diseases [16,17], antidiabetic, anti-hepatoprotective therapy of skin disorders, and personal care formulations. The main fatty acid in MSO, namely oleic acid, has been attributed to promoting a healthy diet, particularly in breast cancer prevention [18]. Furthermore, β-sitosterol is similar to cholesterol and has been demonstrated to inhibit cholesterol absorption into the gut while increasing antioxidant levels, conferring efficacy as an antidiabetic, hypolipidemic, neuroprotective, and chemopreventive agent. Recent experimental investigations on β-sitosterol provide compelling evidence that this compound may serve as a viable supplement in the battle against life-threatening illnesses [18]. The different attributes of Moringa oleifera seed oil enable its utilization in various industrial, medicinal, and pharmaceutical applications.

Due to MSO nanoemulsion's usefulness during food processing, nanoemulsions have been included in various food systems [19, 20] Adding oil-in-water nanoemulsion may increase dairy products' nutritional value [21].To improve stabilizing and emulsifying functions, sodium caseinate (NaCas) is often used to develop delivery systems [22-24]. Therefore, this study aimed to prepare a nanoemulsion with the best ratio from Na Cas and investigate the quality properties of a nanoemulsion made from sodium caseinate and moringa seed oil, as well as the potential application of this nanoemulsion in the production of functional ice cream and a172ffect on ice cream properties.

## 2. Materials and Methods

### *2.1. Materials*

Bovine sodium caseinate was obtained from Sigma/Aldrich (St. Louis, MO, USA). Fresh buffalo skim milk, and cream were obtained from the Dairy Industry Unit, Animal Production Research Institute, Ministry of Agriculture (Dokki, Cairo, Egypt) with an average composition of 10% total solids, 4.15% protein, 0.79% ash, and 0.15% acidity. skim milk "DAIRYAMERICA, Inc. California, USA" (36% protein, 51% lactose, 1.2% fat, 8.2% minerals), was obtained from Giza local market. Other chemicals were obtained from Sigma-Aldrich or Thermo Fisher Scientific, Pittsburgh, PA. All chemicals used were analytical grade.

### *2.2. Methods*

#### *2.2.1. Nanoemulsion preparation*

Nanoemulsion was created with a little modification, a two-step according to Mahdi Jafari et al. [20]. An aqueous phase containing the emulsifier NaCas (5 and 10%) was dissolved in distilled water by stirring at 60 °C and moringa seed oil (MSO) at 5 and 10% concentrations to create the O/W emulsion. A rotor/stator mixer (Polytron, Brinkmann Instruments, ON, Canada) was used for 2 min at 15000 rpm. To further homogenize the mixture, the nanoemulsions were subjected to ultrasonication using 160 W powers, 40 kHz frequency, and 60% pulse (Sonic Vibra Cell USA) for 10 minutes at 5-s time intervals. For the purpose of minimizing temperature rises brought on by ultrasonication, the sample container was placed within a larger glass beaker that was ice-filled [25]. The final emulsions were lyophilized to get the freeze-dried powder (80 °C and 10 mm Hg, LYPHLOCK, Labconco, Kansas City, MO). The obtained nanoemulsions were transferred into plastic cups and sealed, then subjected to freezing at a temperature of -20 °C for application.

*2.2.2. Creaming stability*

Creaming in nanoemulsions was evaluated by putting nanoemulsions (10 ml) into the sterile graduated test tube. Following the advised storage duration, the cream layer volume was determined using the test tube's graduations, and the creaming percentage was determined using the following:

$$\text{Cream\%} = \frac{\text{Cream volume (ml)}}{\text{Sample volume (10 ml)}} \times 100$$

*2.2.3. Nanoemulsion Morphology*

The preparation of MSO nanoemulsions was carried out to evaluate images by transmission electron microscopy (TEM). All samples were diluted at a ratio of 1:100 (v/v) ratios using deionized water. A small amount of the diluted sample was dropped onto the electron microscopy grid of the holey. It was allowed to sit for 1 minute, following which a solution of phosphotungstic acid (2% at pH 7.2) was added. The grid underwent an air-drying process and was then examined via transmission electron microscopy (TEM) utilizing a JEOL JEM-1400 plus TEM instrument. The accelerating voltage was 100 kV, and the magnification was set to 200,000 x.

*2.2.4. Antioxidant activity*

By mixing 2 ml of ice cream sample with 2 ml of 0.1 mM 1,1-diphenyl-2-picrylhydrazyl(DPPH) dissolved in 95% methanol, the activity of DPPH to scavenge free radicals on the MSO and NMSO was evaluated. After being vigorously agitated for 10 seconds, the liquid was left in a dark place for 30 min. The absorbance of the sample was measured at 517 nm, and the DPPH solution without the sample served as a blank. According to Duan et al. [26], the following formula was used to calculate the percentage of DPPH scavenging activity:

$$\text{Scavenging activity of DPPH (\%)} = \frac{(\text{A balnk} - \text{A Sample})}{\text{A balnk}} \times 100$$

Where, ($A_{sample}$) = absorbance of the DPPH solution after the reaction with the sample; ($A_{blank}$) = absorbance of the DPPH solution without sample.

*2.2.5. Ice cream making*

Nanoemulsion was utilized in the production of ice cream to facilitate the effective delivery of omega-3 and polyphenols in MSO. The fresh buffalo skim milk, skim milk powder, cream, sugar, and Lacta 9050 produced basic ice cream blends. The blend comprised 10.5% fat, 10.0% milk solids non-fat, 14.0% sugar, and 0.10% stabilizer. The composite was partitioned into three equitableness segments, whereby the initial fraction was devoid of MSO to establish a reference sample (C). T1 was created by replacing 3% cream with free MSO, and T2 was prepared by replacing 3% cream and 3% skim milk powder with 6 g MSO-nanoemulsions powder (which contains 3g MSO and 3g NaCas). Then, in the preheating step at 65°C and through the mixing of all constituent components, the resulting mixture underwent homogenization via a laboratory homogenizer (EURO TURRAXT 20b, IKA Lobo Technique 27000 min G1). Subsequently, the homogenate was pasteurized at 81°C, cooled, and subjected to an overnight ageing process at a temperature of 5±1°C. Before freezing in a batch freezer (Staff Ice System, BTM 10, Rimini Italy), a 0.5% concentration of vanilla was incorporated into the pre-existing mixture of aged ice cream. The obtained ice cream was transferred into plastic cups and sealed, then subjected to freezing at a temperature of -20 °C for 24 hours before analysis. Each batch was subjected to three replicates.

*2.2.6. Physicochemical characteristics of ice cream mixture*

The chemical composition of the ice cream mixture of total solids, fat content, protein, and ash was determined according to AOAC (2016). The pH values were determined by a laboratory pH meter equipped with a glass electrode (HANNA, Instrument, China), and acidity content was determined by adding 0.1N sodium hydroxide to the ice cream sample with the phenolphthalein endpoint [28].

The whipping ability of the ice cream mixtures was determined by a Heidolph No. 50 111 mixer equipped with 2.6 cm blades of Type RZRI, Germany [29]. The calculation of Overrun was performed on all the formulated ice cream samples using the weight-volume method as outlined by Adapa et al. [27].

*2.2.7. Properties of ice cream*

The ice cream's melting characteristics were determined by the methodology described by Soliman and Shehata [30]. The ice cream samples (40 g), at a temperature of -25°C, were placed on a 1 mm stainless steel mesh over a beaker, and the amount of ice cream drained into the beaker was weighted every 10 min until total loss of structure. Then, melted weight (%) was plotted against the time (minutes).

*2.2.8. Sensory acceptability*

A taste panel of 21 staff members from the Food Science and Technology department at Al-Azhar University, Egypt, evaluated the ice cream samples based on appearance, melting quality, body and texture, and flavour. The sensory evaluation of the ice cream was conducted utilizing the nine-point hedonic scale, which spans from extremely favourable (9 points) to neutral like or dislike (5 points) to extremely unfavourable (1 point), as per the methodology outlined by Soliman and Shehata [30]

*2.2.9. Statistical analysis*

The means were compared using the software SPSS version 15.0 (SPSS Inc. Chicago, Illinois) through Analysis of Variance (ANOVA) and Duncan's multiple comparison procedure. Statistical significance was established using a probability threshold of $p<0.05$.

## 3. Results

### *3.1. Nanoemulsion characteristics*

The process of formulating plays a crucial role in the manufacturing of nanoemulsion, as it enables the attainment of specific parameters such as small particle size (ranging from 20-200 nm in diameter) and low PDI (<0.5). Furthermore, it is imperative to consider the particle size as it plays a pivotal role in evaluating the product's stability, visual characteristics, bioaccessibility, and final texture [31, 32].

Four distinct formulations, namely T1, T2, T3, and T4, were utilized to generate nanoemulsions. These formulations' particle sizes exhibited significant differences ($p < 0.5$), as evidenced by the values of 125.80, 178.40, 160.65, and 167.50, nm, respectively, as presented in Table 1. T1 had the smallest particle size (125.80 nm) when compared to the other three nanoemulsions and had a narrow size distribution, as shown by the PDI values (0.390). MSO nanoemulsions have a unimodal distribution.

Moreover, the incorporation of oil resulted in a rise in particle dimensions, as observed in T2. The T2 sample exhibited a particle size of 178.40 nm, the largest of the tested samples. However, it demonstrated a narrow particle size distribution, as evidenced by the low values of PDI (0.329). The findings were consistent with the prior investigation, indicating that the β-carotene nanoemulsions generated utilizing elevated emulsifier concentrations exhibited a greater particle size [33]. This phenomenon may be attributed to the emulsifiers encapsulating the nanoparticles within the nanoemulsion, leading to the possibility of unfavourable particle size upon increasing the concentration.

Table 1. Effect of NaCas on MSO nanoemulsions droplet size, zeta potential, and polydisperisty index

| samples | Particle size (nm) | PDI | Zeta potential |
|---|---|---|---|
| **T1** | **$125.80^{d} \pm 9.40$** | **$0.390^{b} \pm 0.013$** | **$-34.1^{d} \pm 5.68$** |
| **T2** | **$178.40^{a} \pm 12.30$** | **$0.334^{c} \pm 0.012$** | **$-35.5^{c} \pm 5.00$** |
| **T3** | **$160.65^{c} \pm 13.40$** | **$0.424^{a} \pm 0.015$** | **$-37.0^{b} \pm 7.81$** |
| **T4** | **$167.50^{b} \pm 15.30$** | **$0.329^{d} \pm 0.013$** | **$-41.9^{a} \pm 5.56$** |

T1: 5% NaCas and 5% MO; T2: 5% NaCas 10% MO; T3: 10% NaCas 5% MO; T4: 10% NaCas 10% MO.

In addition, the Zeta potential was utilized to determine the stability of the nanoemulsions by measuring the intensity of the electrical repulsion force between particles. The Zeta potential values of four nanoemulsions samples, T1, T2, T3, and T4, were measured and recorded as (-34.10, -35.50, -37.00, and -41.90), respectively. Theoretically, a high zeta potential value indicates excellent stability since it inhibits tiny droplets from flocculating and forming close interactions. Additionally, this property is influenced by the nanoparticle composition and dispersion medium [34]. The zeta potential is calculated using the electrophoretic mobility corresponding to the edge of the surrounding liquid layer related to the moving particles in the medium [34]. This study's findings indicated

significant differences ($p < 0.5$) across the samples, indicating that the stability of MSO nanoemulsions was impacted by emulsifier concentrations with a concentration of 10% oil. Consequently, the T4 sample had the greatest zeta potential value, indicating the most potent electrostatic attraction between droplets, leading to a stable emulsion.

The PDI (Polydispersity index) indices have been determined as 0.390, 0.334, 0.424, and 0.329, respectively. The nanoemulsion prepared from 10% MSO and 10% NaCasas a shell (T4) exhibited the lowest polydispersity index (PDI) and highest electronegativity. The observed outcome may be attributed to the high electronegativity of sodium caseinate and the hydrophobic linkages of MSO. [35, 36] discovered that in the production of O/W nanoemulsions, the utilization of sodium caseinate, which a high molecular weight (10-50 kDa), high electronegativity, and gelatinization capacity, along with a thick interfacial layer, led to an increase in the attraction between oil and NaCas, which in turn reduced flocculation and aggregation, resulting in improved emulsifying stability [37] has reported that the stability of emulsions is significantly influenced by the electronegativity of sodium caseinate and the thickness of the emulsion interface.

As per the findings of [35], augmentation of the surface charge of the emulsion resulted in the enhancement of repulsive forces among the droplets. This, in turn, impeded flocculation and accumulation, thereby promoting the stability of the oil-water interface.

### *3.2. Creaming stability*

The measurements of creaming stability for the various MSO nanoemulsion formulations, including T1, T2, T3, and T4, revealed significant variances ($p < 0.5$). The 10% emulsifiers (NaCas) and 10% MSO used to create the nanoemulsion T4 showed greater stability, and no creaming development was seen after 7 days of storage at 25 °C. Significant differences ($p < 0.5$) and decreased stability after storage were seen in T1 and T2 nanoemulsion samples made with 5% emulsifier (NaCas) and 5% or 10% MSO, respectively. While T4 showed high stability, no creaming separation, and zeta potential -41.9a ± 5.56 mV, the findings of the creaming stability test corresponded well with zeta potential. Huck-Iriart et al. [38] found that nanoemulsion lipid was stable when no sediment or creaming was seen in nanoemulsions with a zeta potential value higher than -40 mV. The T3 sample, made with 10% and 5% NaCas, showed little stability throughout storage time. As a result of the nanoemulsion travelling through caseinate-coated droplets, depletion flocculation brought on by nonabsorbed caseinate of submicelles, utilising a high concentration of NaCas, may have a negative effect [38]. Additionally, it was discovered that samples T1 and T2 created with 5% NaCas emulsifier concentration had lower stability than samples T3 and T4 (Figure 1). Similar outcomes were found by Hamed et al. [25], the low emulsifier concentration of 5% (w/w), which was insufficient to encapsulate the oil droplets effectively and led to destabilisation. Figure 1 demonstrated that after seven days of storage at 25°C, sample T4 had not developed any cream. Consequently, reaching an acceptable concentration close to the saturation surface coverage value is necessary to get nanoemulsions that are the most stable in creaming. The technique delivers sufficient protein to provide excellent surface coverage while stabilising electrostatics in the direction of coalescence and creaming [38].

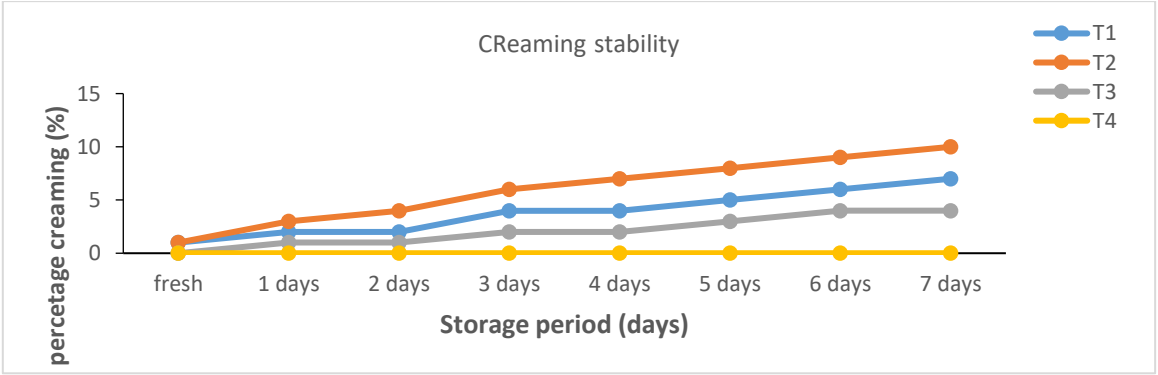


**Figure 1.** Creaming stability of MSO nanoemulsion during storage at 25 ± 2 °C for 7 days

*3.3. Morphology of droplets nanoemulsion*

Transmission electron microscopy (TEM) was employed after the nanoemulsion formulations were created to see the particles' shape. TEM was used to validate particle size and ensure excellent encapsulation of 5 or 10% MSO in two NaCas concentrations of 5% (T1, T2) and 10% (T3, T4) (Figure 2). The micrographs of samples revealed particles with more than 100 nm diameters agree with particle size obtained from the Dynamic laser scattering Zeta-sizer. Particle morphology in all samples was spherical, with a few minor variations. Mohammed et al. [24] reported similar findings, demonstrating that the TEM picture revealed the nanoparticle of Nigella sativa oil nanoemulsion had a spherical form and a size of around 200 nm. Soliman & Nasser [33] reported comparable results for an olive oil nanoemulsion containing various beta-carotene concentrations created using a sodium caseinate emulsifier and displayed spherical forms of different diameters.

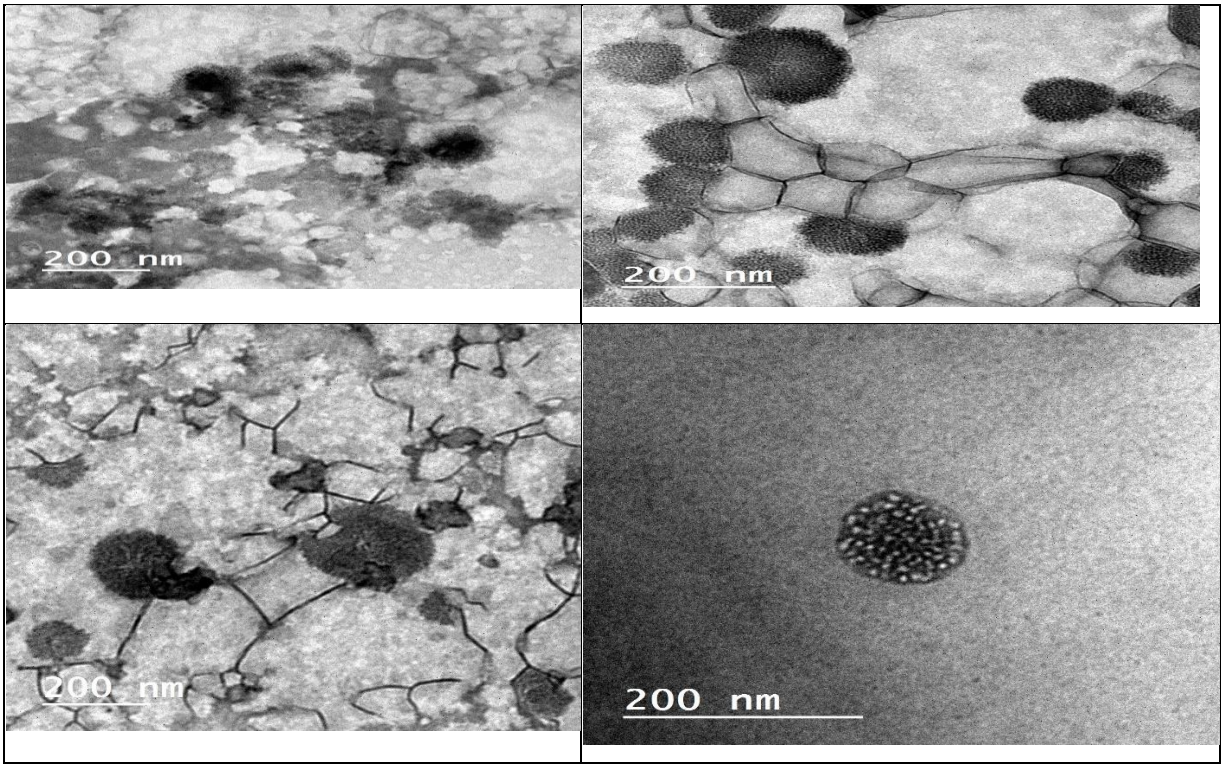


**Figure 2.** Transmission electron micrographs of MSO nanoemulsion

*3.4. Making ice cream incorporating MSO nanoemulsions*

Ice cream is a viscous frozen matrix phase that contains fat globules, air bubbles, and ice crystals [39]. Ice cream typically includes 10% to 16% fat [40]. Based on the nanoemulsion properties results, the optimal ratio of NaCas as an emulsifier and MSO addition (10%NaCas-10% MSO) was chosen. Because results that are more consistent were obtained, the stability of the nanoemulsion formulations was investigated using gravity separation data at 25°C. Nanoemulsions containing 10% NaCas had similar stability values. Because of their low high viscosity and consistency, they were stable during storage. This study used standard processes to compare these study-developed samples with the same fat concentration as ice cream made with nanoemulsion, free MSO, and regular fat content. The control samples were created without free MSO or nanoemulsions. All remaining treatments were prepared by replacing the fat content with the nanoemulsion or free MSO to save ice cream recipes calculated.

*3.5. Physicochemical Properties of ice cream*

One of the most effective techniques for increasing the solubility and bioavailability of nonpolar active compounds is nanoemulsions. The food sector wants these emulsion systems to include lipophilic nutrients in novel food products. Table 2 displays the chemical composition and scavenger activity of ordinary ice cream mix as the control and ice cream supplemented with either free MSO (T1) or MSO nanoemulsion (T2). All ice cream treatments exhibited an entire solids content of 35.40%. The control sample had the highest pH value of 6.75 but no significant change in pH with nanoemulsion addition. Adding 3% free MSO (T1) decreased the pH significantly ($p < 0.05$) to 6.53, whereas (T2) formulated with 3% MSO nanoemulsion reduced the pH to 6.69. Because of the moringa seed oil's high phenolic content. Sagdic et al. [41] found that adding several phenolic compounds such as gallic acid, ellagic acid, and extracts from peppermint and grape seed improved ice cream samples ($p < 0.05$). Hwang et al. [42] discovered that adding grape wine high in phenolic components to ice cream produces a pH value owing to the acidic substance.

The total proteins of plain ice cream (control) and ice cream incorporated with 3% free MSO (T1) were 7.35%, which increased to 8.74% for ice cream incorporated with MSO nanoemulsion due to adding 6 g of powdered MSO nanoemulsion containing 3 3 g NaCas (97% protein). This increase in total proteins was significant ($p < 0.05$) compared to the control and T1 mixes. These results agree with Soliman and Shehata [30] using curcumin whey protein isolate conjugation.

**Table 2.** Physicochemical of Ice cream incorporated with free MSO or MSO nanoemulsion

| **Parameters** | Control | T1 | T2 |
|---|---|---|---|
| **Total solids (%)** | $35.40^{a} \pm 0.75$ | $35.40^{a} \pm 0.95$ | $35.45^{a} \pm 1.03$ |
| **Total Protein (%)** | $7.25^{a} \pm 0.05$ | $7.25^{a} \pm 0.10$ | $8.60^{ab} \pm 0.15$ |
| **Fat (%)** | $10.55^{a} \pm 0.05$ | $10.60^{a} \pm 0.05$ | $10.30^{a} \pm 0.10$ |
| **Ash (%)** | $1.10^{a} \pm 0.03$ | $1.18^{a} \pm 0.07$ | $1.14^{a} \pm 0.03$ |
| **pH** | $6.75^{a} \pm 0.01$ | $6.53^{b} \pm 0.02$ | $6.69^{ab} \pm 0.03$ |
| **Acidity (%)** | $0.33^{b} \pm 0.03$ | $0.40^{a} \pm 0.04$ | $0.37^{ab} \pm 0.03$ |
| **Antioxidant Activity (%)** | $33.54 \pm 3.12$ | $44.59 \pm 2.01$ | $61.35 \pm 2.75$ |
| **Overrun %** | $61.00 \pm 2.95$ | $61.97 \pm 3.45$ | $88.25 \pm 2.75$ |

The MSO nanoemulsion-infused ice cream mix had the highest DPPH radical scavenging activity (61.35%), followed by free MSO-infused ice cream (44.59%) and the control mix (33.54%). According to [9], ice cream enriched with M. oleifera oil had increased antioxidant activity.

*3.5.1. Overrun*

According to Marshall et al. [45], the overrun of ice creams is the measuring of the air content of cells or small bubbles that must be properly generated and maintained during manufacture. Table 2 displays the overrun values for ice cream made with nanoemulsions of MSO. Compared to the control and T1 sample, the overrun value of T2 ice cream made with 3% MSO nanoemulsion was greater. The high percentage of overrun demonstrates great stability and stiffness of the froth of the formulation of ice cream. The sodium caseinate enhanced the overrun value from 61.00 to 88.25%, owing to its functional capabilities as stabilizing and emulsifying agent. MSO nanoemulsion was an additional emulsifier and stabilizer agent that enhanced the final product's properties. The overrun values for the three formulations are consistent with Mohammed et al. [30],whose finings for ice cream containing Nigella sativa oil nanoemulsion at four ratios (0 control, 3, 5, and 10%) ranged from 66.7 to 75.4%.

*3.5.2. Whipping ability (%)*

Whipping ability was improved by sodium caseinate in all their beneficial ways. The control ice cream and ice cream mixed with free MSO had no

significant change in volume and whipping times between them, as illustrated in Fig. 4. The T2 combination enhanced with MSO nanoemulsion showed the most crucial whipping ability after 5 minutes. It continued after 10 and 20 minutes ($p < 0.05$) compared to the control and T1 mixes. There was no noticeable change between the control mix and T1 supplemented with free MSO at 5, 10, or 20 minutes (p 0.05). Qu & Zhong [46] found that adding WPI-MD conjugate may have boosted the air incorporation within the frozen milk matrix by increasing the binding in the air-cell membrane.

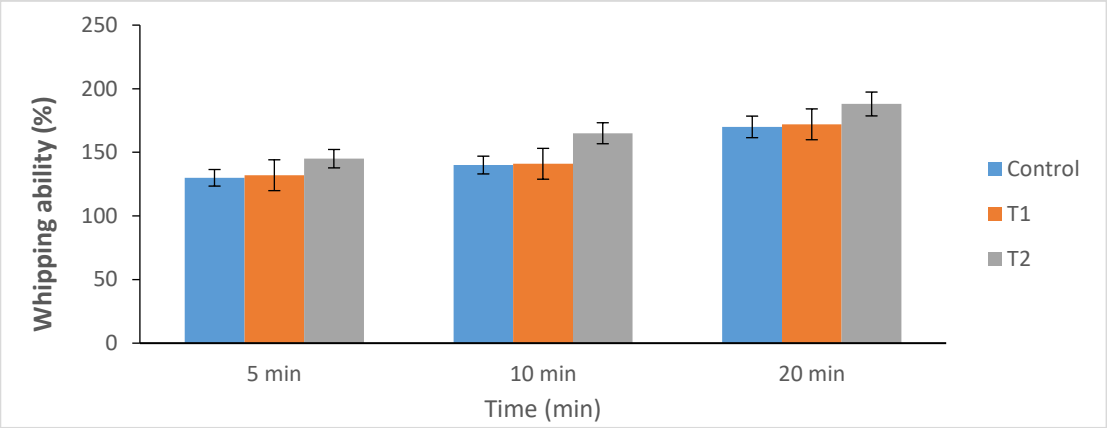


**Figure 1**. Whipping ability (%)of ice cream incorporated with MSO nanoemulsion

*3.5.3. Melting resistance*

The physical property of melting resistance holds significant importance in ice cream products. The present investigation enhanced the resistance of ice cream melting by utilizing MSO nanoemulsion (T2) owing to its remarkable stability at diverse concentrations, as demonstrated in Table 2. The order of the ice cream's melting speeds was as follows: T2 < control < T1, with significant differences ($p < 0.05$), as indicated in Figure 3. After 145 min on stainless steel mesh, the T2 formulation melted, but the control ice cream formulation melted after 115 min. The T1 ice cream exhibited an earlier melting after 2-3 minutes than the remaining two samples (control and T2). Furthermore, it demonstrated the highest melting rate, particularly after 100 min. High melting resistance was seen when ice cream formulations utilized MSO nanoemulsion. This is because the components in the nanoemulsion made fromNaCas have certain valuable qualities. Mohammed et al. [24] claim that Nigella sativa oil was effectively encapsulated and utilized in the production of ice cream using a mixture of sodium caseinate and maltodextrin and has high melting resistance.

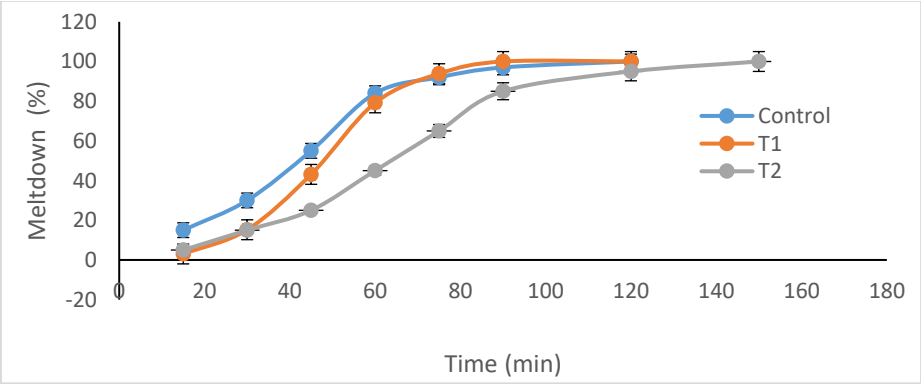


**Figure 2.** Melting resistance of ice cream incorporated with free MSO and MSO nanoemulsion

According to Qu and Zhong [46], protein conjugation with reducing saccharides such as maltodextrins (MD) may enhance the stabilizing and emulsifying properties of oil/water (O/W) emulsions. As an emulsifier, sodium caseinate (NaCas) is primarily used to construct delivery systems. Zhang et al. [47], found that sodium caseinate-maltodextrin conjugates significantly improved the freeze-thaw stability of oil-in-water emulsions compared to control proteins and protein mixtures containing sugar. Granger et al.[48] also described how to make ice cream comprising refined and hydrogenated coconut oil and refined palm oil in addition to the emulsifiers saturated monoglycerides and diglycerides. The researchers found that the emulsifier's characteristics and the ice cream formulation had the most influence on melting times, with the kind of oil having the most negligible impact. Although there is a noticeable variation in the melting speeds after the structure starts to deform, it is exceedingly unlikely that there would be any detectable variance in the time of the first drop of the samples [49]. According to Nazaruddin et al. [50], the air content in ice cream influences how quickly it melts. As an example, the amount of the ice cream overrun is raised. A more significant overrun results in a bigger and softer proportion of ice cream melting. This research finds that the higher overrun (88.25 g/100 g) is associated with the T2 ice cream's increased melting resistance, which supports the hypothesis.

*3.5.4. Sensory evaluation*

The sensory assessment used a nine-point Hedonic Scale ranging from strong dislike (1) to very like (9). The flavuor of the ice cream (T1) prepared with 3% free MSO was evaluated as satisfactory by panelists. As a control ice cream, ice cream (T2) formulated with 3% MSO nanoemulsion demonstrated great acceptance. Dhawi et al. [35], found that yoghurt enhanced with moringa seed flour has poor acceptance owing to its MSO character.

MSO nanoemulsions exhibit white hues with remarkable brightness and don't influence the colour of ice cream sample (Figure 5). However, no significant variations in colour were seen between the control and MSO-enriched samples. The body and texture values for MSO nanoemulsions samples are higher than ice cream with Free MSo and equal to the control.

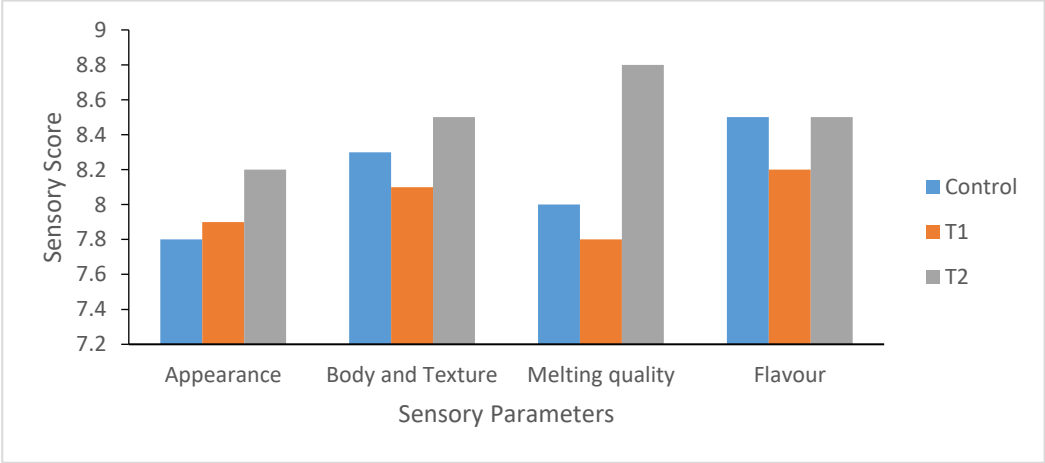


**Figure 5.** Sensory evaluation of ice cream fortified with MSO nanoemulsion

## 4. Conclusion

This work demonstrated that MSO might be effectively made as a nanoemulsion. Using 10% NaCas and 10% MSO, a typical MSO nanoemulsion had an adequate particle size, high stability, and good shape droplet. The findings showed that adding MSO nanoemulsion to ice cream improved its physicochemical properties and sensory acceptability. The sensory assessment of the ice cream samples indicated that the ice cream (T2) formed with 6% MSO nanoemulsion was the most flavoured of the three formulations. It is possible to infer that MSO in nanoemulsion form may be used in the ice cream

sector and food industries with high stability and acceptability.

## 5. References


1. USDA (2012). United States Department of Agriculture .National Agriculture Statistics Service, 16 July 2012. US Department of Agriculture.
2. Kilara, A., & Chandan, R. C. (2013). Frozen dairy foods. Milk and Dairy Products in Human Nutrition: Production, Composition and Health, 435-457.
3. Soukoulis, C., Fisk, I. D., & Bohn, T. (2014). Ice cream as a vehicle for incorporating health-promoting ingredients: Conceptualization and overview of quality and storage stability. *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 627-655.
4. Legassa, O. (2020). Ice Cream Nutrition and Its Health Impacts.
5. Çam, M., İçyer, N. C., & Erdoğan, F. (2014). Pomegranate peel phenolics: Microencapsulation, storage stability and potential ingredient for functional food development. *LWT-Food Science and Technology*, *55*(1), 117-123.
6. Alfaro, L., Hayes, D., Boeneke, C., Xu, Z., Bankston, D., Bechtel, P. J., & Sathivel, S. (2015). Physical properties of a frozen yogurt fortified with a nanoemulsion containing purple rice bran oil. *LWT-Food Science and Technology*, *62*(2), 1184-1191.
7. Danesh, E., Goudarzi, M., & Jooyandeh, H. (2017). Effect of whey protein addition and transglutaminase treatment on the physical and sensory properties of reduced-fat ice cream. *Journal of Dairy Science*, *100*(7), 5206-5211.
8. Sun-Waterhouse, D., Edmonds, L., Wadhwa, S. S., & Wibisono, R. (2013). Producing ice cream using a substantial amount of juice from kiwifruit with green, gold or red flesh. *Food Research International*, *50*(2), 647-656.
9. Ullah, R., Nadeem, M., & Imran, M. (2017). Omega-3 fatty acids and oxidative stability of ice cream supplemented with olein fraction of chia (Salvia hispanica L.) oil. *Lipids in health and disease*, *16*(1), 1-8.
10. Gowda, A., Sharma, V., Goyal, A., Singh, A. K., & Arora, S. (2018). Process optimization and oxidative stability of omega-3 ice cream fortified with flaxseed oil microcapsules. *Journal of food science and technology*, *55*, 1705-1715.
11. Güven, M., Kalender, M., & Taşpinar, T. (2018). Effect of using different kinds and ratios of vegetable oils on ice cream quality characteristics. *Foods*, *7*(7), 104.
12. Boukandoul, S., Santos, C. S., Casal, S., & Zaidi, F. (2019). Oxidation delay of sunflower oil under frying by moringa oil addition: more than just a blend. *Journal of the Science of Food and Agriculture*, *99*(12), 5483-5490.
13. Pereira, F. S. G., de Brito Neto, E. X., Wei, S., Galvão, C. C., de Lima, V. F., da Silva, V. L., & de Lima Filho, N. M. (2016). Produção de biodiesel metílico com óleo purificado de Moringa oleifera lamarck. *Revista Virtual de Química*, *8*(3), 873-888.
14. Danesh, E., Goudarzi, M., & Jooyandeh, H. (2017). Effect of whey protein addition and transglutaminase treatment on the physical and sensory properties of reduced-fat ice cream. *Journal of Dairy Science*, *100*(7), 5206-5211.
15. Suryadevara, V., Doppalapudi, S., LC, S. R., Anne, R., & Mudda, M. (2018). Formulation and evaluation of anti-inflammatory cream by using Moringa oleifera seed oil. *Pharmacognosy Research*, *10*(2).
16. Middleton, E., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological reviews*, *52*(4), 673-751.
17. Dinesha, B. L., Nidoni, U., Ramachandra, C. T., Naik, N., & Sankalpa, K. B. (2018). Effect of extraction methods on physicochemical, nutritional, antinutritional, antioxidant and antimicrobial activity of Moringa (Moringa oleifera Lam.) seed kernel oil. *Journal of Applied and Natural Science*, *10*(1), 287-295.
18. Gharsallah, K., Rezig, L., & Mahfoudhi, N. (2022). Beneficial Effects of Moringa oleifera Seed Oil Bioactive Compounds. In *Handbook of Research on Advanced Phytochemicals and Plant-Based Drug Discovery* (pp. 268-291). IGI Global.
19. Anton, N., Gayet, P., Benoit, J. P., & Saulnier, P. (2007). Nanoemulsions and nanocapsules by the PIT method: an investigation on the role of the

temperature cycling on the emulsion phase inversion. *International Journal of Pharmaceutics*, *344*(1-2), 44-52.

20. Mahdi Jafari, S., He, Y., & Bhandari, B. (2006). Nanoemulsion production by sonication and microfluidization—a comparison. *International journal of food properties*, *9*(3), 475-485.
21. Cheong, A. M., Tan, K. W., Tan, C. P., & Nyam, K. L. (2016). Kenaf (Hibiscus cannabinus L.) seed oil-in-water Pickering nanoemulsions stabilised by mixture of sodium caseinate, Tween 20 and β-cyclodextrin. *Food Hydrocolloids*, *52*, 934-941.
22. Mohammed, N. K., Meor Hussin, A. S., Tan, C. P., Abdul Manap, M. Y., & Alhelli, A. M. (2017). Quality changes of microencapsulated Nigella sativa oil upon accelerated storage. *International Journal of Food Properties*, *20*(sup3), S2395-S2408.
23. Mohammed, N. K., Tan, C. P., Abd Manap, M. Y., Muhialdin, B. J., & Meor Hussin, A. S. (2019). Production of functional non-dairy creamer using Nigella sativa oil via fluidized bed coating technology. *Food and Bioprocess Technology*, *12*, 1352-1365.
24. Mohammed, N. K., Muhialdin, B. J., & Meor Hussin, A. S. (2020). Characterization of nanoemulsion of Nigella sativa oil and its application in ice cream. *Food science & nutrition*, *8*(6), 2608-2618.
25. Hamed, S. F., Soliman, T. N., Hassan, L. K., & Abo-Elwafa, G. (2019). Preparation of functional yogurt fortified with fish oil-in-water nanoemulsion. *Egyptian Journal of Chemistry*, *62*(Special Issue (Part 1) Innovation in Chemistry), 301-314.
26. Duan, X., Li, M., Ma, H., Xu, X., Jin, Z., & Liu, X. (2016). Physicochemical properties and antioxidant potential of phosvitin–resveratrol complexes in emulsion system. *Food Chemistry*, *206*, 102-109.
27. Adapa, S., Dingeldein, H., Schmidt, K. A., & Herald, T. J. (2000). Rheological properties of ice cream mixes and frozen ice creams containing fat and fat replacers. *Journal of dairy science*, *83*(10), 2224-2229.
28. Arbuckle, W.S., 1986. Ice Cream. 4th Edn., Springer, ISBN: 978- 1-4615-7222-0 Pages: 483.
29. Baer, R. J., Krishnaswamy, N., & Kasperson, K. M. (1999). Effect of emulsifiers and food gum on nonfat ice cream. *Journal of Dairy Science*, *82*(7), 1416-1424.
30. Soliman, T. N., & Shehata, S. (2021). Preparation and Characterization of Functional Ice Milk Incorporated with Curcumin Nano-Encapsulated in Conjugated Whey Protein-Maltodextrin. *International Journal of Dairy Science*, 16, 58-66.
31. Acosta, E. (2009). Bioavailability of nanoparticles in nutrient and nutraceutical delivery. *Current opinion in colloid & interface science*, *14*(1), 3-15.
32. McClements, D. J., & Rao, J. (2011). Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Critical reviews in food science and nutrition*, *51*(4), 285-330.
33. Soliman, T. N., & Nasser, S. A. (2022). Characterization of carotenoids double-encapsulated and incorporate in functional stirred yogurt. *Frontiers in Sustainable Food Systems*, *6*, 979252.
34. Wu, L., Zhang, J., & Watanabe, W. (2011). Physical and chemical stability of drug nanoparticles. *Advanced drug delivery reviews*, *63*(6), 456-469.
35. Charoen, R., Jangchud, A., Jangchud, K., Harnsilawat, T., Naivikul, O., & McClements, D. J. (2011). Influence of biopolymer emulsifier type on formation and stability of rice bran oil-in-water emulsions: whey protein, gum arabic, and modified starch. *Journal of food science*, *76*(1), E165-E172.
36. Ozturk, B., & McClements, D. J. (2016). Progress in natural emulsifiers for utilization in food emulsions. *Current Opinion in Food Science*, *7*, 1-6.
37. Pilosof, A. M. (2017). Potential impact of interfacial composition of proteins and polysaccharides stabilized emulsions on the modulation of lipolysis. The role of bile salts. *Food Hydrocolloids*, *68*, 178-185.
38. Huck-Iriart, C., Álvarez-Cerimedo, M. S., Candal, R. J., & Herrera, M. L. (2011). Structures and stability of lipid emulsions formulated with sodium caseinate. *Current Opinion in Colloid & Interface Science*, *16*(5), 412-420.
39. Eisner, M. D., Wildmoser, H., & Windhab, E. J. (2005). Air cell microstructuring in a high viscous ice cream matrix. *Colloids and Surfaces A:*

*Physicochemical and Engineering Aspects*, *263*(1-3), 390-399.

40. Goff, H. D. (1997). Colloidal aspects of ice cream—a review. *International dairy journal*, *7*(6-7), 363-373.
41. Sagdic, O., Ozturk, I., Cankurt, H., & Tornuk, F. (2012). Interaction between some phenolic compounds and probiotic bacterium in functional ice cream production. *Food and Bioprocess Technology*, *5*, 2964-2971.
42. Hwang, J. Y., Shyu, Y. S., & Hsu, C. K. (2009). Grape wine lees improves the rheological and adds
43. antioxidant properties to ice cream. *LWT-Food Science and Technology*, *42*(1), 312-318.
44. Çelİk, Ş., Cankurt, H., & Doğan, C. (2010). The effect of saffron addition on some properties of plane ice-cream. *GIDA-Journal of Food*, *35*(1), 33-39.
45. Jakubczyk, E., & Niranjan, K. (2006). Transient development of whipped cream properties. *Journal of food engineering*, *77*(1), 79-83.
46. Marshall, R. T., Goff, H. D., & Hartel, R. W. (2003). *Ice cream*. Springer Science & Business Media.
47. Qu, B., & Zhong, Q. (2017). Casein-maltodextrin conjugate as an emulsifier for fabrication of structured calcium carbonate particles as dispersible fat globule mimetics. *Food Hydrocolloids*, *66*, 61-70.
48. Zhang, Z., Wang, X., Yu, J., Chen, S., Ge, H., & Jiang, L. (2017). Freeze-thaw stability of oil-in-water emulsions stabilized by soy protein isolate-dextran conjugates. *LWT*, *78*, 241-249.
49. Granger, C., Langendorff, V., Renouf, N., Barey, P., & Cansell, M. (2004). Impact of formulation on ice cream microstructures: an oscillation thermo-rheometry study. *Journal of dairy science*, *87*(4), 810-812.
50. Maurya, A., Gupta, S., & Srivastava, S. K. (2011). Preparative isolation of bioactive nitrile glycoside "Niazirin" from the fruits of Moringa oleifera using fast centrifugal partition chromatography. *Separation Science and Technology*, *46*(7), 1195-1199.
51. Nazaruddin, R., Syaliza, A. S., & Wan Rosnani, A. I. (2008). The effect of vegetable fat on the physicochemical characteristics of dates ice cream. *International journal of dairy technology*, *61*(3), 265-269.
52. Dhawi, F., El-Beltagi, H. S., Aly, E., & Hamed, A. M. (2020). Antioxidant, antibacterial activities and mineral content of buffalo yoghurt fortified with fenugreek and Moringa oleifera seed flours. *Foods*, *9*(9), 1157.