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Preparation and characterization Nanoemulsion Moringa Oil by Whey Protein and Application in Ice Cream as a Food Model

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Abstract

Moringa seed oil contains several bioactive compounds. These compounds are rich in antioxidants and thus endowed with favorable protective and therapeutic properties. The current study aimed to investigate the prepare a nanoemulsion using whey proteins, with a study of its properties and its application as a food model in ice cream. Expressed on dry weight basis, the seeds exhibited a moisture content of 7.20 ± 0.75, whereas contents of proteins, fiber, ash, fat, and total carbohydrate were25.35 ± 0.15 %, 7.95 ± 0.25%, 5.57 ± 0.23%, 44.33 ± 1.09%, 17.85 ± 0.72%, respectively. Oleic acid was the main fatty acid (C18:1) 36.63%. Oil-in-water nanoemulsions were prepared by ultrasonic homogenization. This emulsion is created using ultrasound, which generates mechanical vibration and cavitation to produce nanoemulsion. Particle size, polydispersity index (PDI), and zeta potential (surface charge) were measured as indicators of the influence of concentrations of whey protein isolate and M. Oleifera oil on the stability of the generated nanoemulsions. From the results, the PDI decreased with increasing WPI but increased with increasing moringa oil. 10% WPI was compatible with incorporated in ice cream. Moringa seed oil contains several bioactive compounds. These compounds are rich in antioxidants and thus endowed with favorable protective and therapeutic properties. We concluded to produce functional ice cream fortified with moringa seed oil-based nanoemulsion rich-omega-3. The prepared ice cream fortified with MO-based nanoemulsions showed good physicochemical characteristics and good sensory attributes.

Key words: whey protein isolate, nanoemulsion, Moringa oil, ice cream, particle size, zeta potential.

INTRODUCTION

Moringa oleifera is one of the various medicinal plants that can be used as a functional and natural food additive. It has been used for centuries, concerning its use in more than 80 countries, and researches about its nutritional values has been carried out since 1970. In 1998, the World Health Organization (WHO) promoted this plant as an alternative supplement to treat malnutrition (Mahmood et al., 2010). The oil obtained from the seed kernels of Moringa oleifera is yellowish brown, semi-solid, with a low odor of bitter almonds. M. oleifera seed kernels possess a significant oil content (up to 40%), a high proportion of fatty acids (oleic acid > 70%), and a remarkable resistance to oxidative degradation. Nutraul enough, this rich oil profile makes the Moringa seeds ideal for human ingestion and commercial utilization (Anwar et al.,2005). Moringa seed oil contains several bioactive compounds. These compounds are rich in antioxidants and thus endowed with favorable protective and therapeutic properties. Extracting oil from M. oleifera seeds might effectively contribute to its application in the cosmetic, pharmaceutical, and medicinal industries(Karima et al.,2020).

Whey protein has antioxidant, opiate, immunomodulatory, and anti-diabetic properties. Whey proteins isolate is an important emulsifier in food emulsions (Adjonu et al., 2014; Li et al., 2018) and is also the source of bioactive peptide molecules, which are beneficial for promoting good health in humans. These peptides are considered safe and healthy compounds, easily absorbed by the human body, and used as functional and nutraceutical agents (Yadav et al., 2015).

Moreover, nanoemulsion systems are excellent for delivering bioactive compounds in foods and

beverages. Oil-in-water nanoemulsions have distinct physicochemical properties from conventional ones, including smaller droplet sizes, a larger surface area, enhanced stability, distinctive inter-facial and bulk rheological properties, and weakly scattered light (Kumar et al., 2018). These characteristics make nanoemulsions ideal for incorporating bioactive compounds into foods, beverages, and gels (Kumar et al., 2018).

Nanoemulsions also aid in the transport and controlled delivery, encapsulation, bioavailability, and bioaccessibility of lipophilic and phytophenolic food components, such as β-carotene, vitamin E, polyunsaturated fatty acids, essential oils, and antimicrobial compounds. Moreover, nanoencapsulation of bioactive peptides provides a pathway for including bioactive peptides in foods.

The current work investigated the emulsifying properties of WPI to moringa seed oil as nanoemulsions. Also, incorporating these emulsions in functional ice cream demonstrates a food system.

MATERIALS AND METHODS

Moringa oleifera seeds were purchased from the moringa unit, National research centre, Giza, Egypt.Methods

Oil content

The AOAC standards technique was used to determine the oil content (AOAC, 2000). Using a coffee grinder, the dehulled seed was ground into a fine paste. Two g of seed powder were extracted with hexane at 40–60 °C for 8 hours using a Soxhlet extractor equipped with a 500-ml round-bottomed flask and condenser. This was repeated three times, and the oil content was represented as a dry matter percentage.

Preparation of Moringa oleifera seeds oil

The kernels are further dried for a week at room temperature. The seeds were handled carefully for cleanliness. On the first day, the M. oleifera seeds, each weighing five kilograms, were poured into the receiving funnel of the cold press. For the next few minutes, the oil was dripped from the outlet and collected in a container that had previously been weighed, and the oil with the container was weighed and subtracted to express the weight of the oil (1.5 kg).

Content of free fatty acids

The free fatty acid concentration was measured using the AOAC method 940.28 (titration technique) (AOAC, 2000). Alcohol was used to dissolve the samples, which were then neutralized with phenolphthalein. Titration was performed with 0.25 M sodium hydroxide. The titration volume of sodium hydroxide was given as a percentage of free fatty acids, represented as oleic acid.

Density and viscosity

The viscosity and density were determined using an ASTM Method D7042-11 Brookfield viscometer (Model DV IM) (ASTM, 2011). A rotating coaxial cylinder measurement mechanism is used by the Brookfield viscometer. The test specimen is placed in measurement cells that are kept at a constant and known temperature of 20 °C. A U-shaped oscillating sample tube and an electronic excitation and frequency counting device are used in the digital density analyzer. The temperature of the equipment was fixed at 20 degrees Celsius. The syringe was filled with 5 ml of oil sample. The syringe was inserted into the viscometer's input aperture, and 2 ml of oil was injected into the measuring cells before the measurement began without the syringe being removed. When the instrument showed that the judgement was correct, the values were recorded. The measurement was repeated after another 1 ml injection. This was done three times, with the final figure indicating the average of the three readings. This technique was carried out three times.

Fatty acids Composition

The fatty acid composition was measured using DGF-C-VI 10, (2013) in conjunction with DGF-C-VI 11d (2013). The prepared sample was injected into an HP 5890 gas chromatograph (Agilent Technologies Sales & Services GmbH & Co. KG, Waldbronn, Germany) equipped with a CP-Sil 88 capillary column (100 m length, 0.25 mm ID, film thickness of 0.2 m). The temperature programme looked like this: 10-minute isotherm from 155°C to 220°C (1.5 °C/min); injector 250°C; detector 250°C; carrier gas 36 cm/s hydrogen; split ratio 1:50; detector gas 30 mL/min hydrogen; 300 mL/min air; and 30 mL/min nitrogen; manual injection volume less than 1 L. The integration programme calculated the peak areas, and the percentages of fatty acid methyl esters (FAME) were acquired as weight percent by direct internal normalisation.

Preparation of Nanoemulsions

Overnight stirring using a benchtop magnetic stirrer produced solutions with different sodium caseinate or whey protein isolate concentrations (5 and 10% w/w). To prevent the microbial growth, sodium azide (0.02%) was added to the aqueous solution. In order to produce coarse oil-in-water emulsions, moringa oil (5 or 10% w/w) and protein solutions were mixed together for 1 minute at 20000 rpm in a rotor/stator mixer (Polytron, Brinkmann Instruments, ON, Canada). Following this, the nanoemulsions were exposed to ultrasonication with 160 W power, 40 kHz frequency, and 60% pulse (Sonic Vibra Cell USA) for 10 minutes at 5-s time intervals, with the goal of further homogenising the mixture. The sample container was placed within a larger glass beaker filled with ice to reduce temperature increases caused by ultrasonication. All of the emulsions were placed in 120-mL glass bottles and stored at room temperature (25 ±1 °C) for one month. Emulsions have a pH of 6.8–7.0.

Creaming

By transferring nanoemulsions (10 mL) into the sterile graduated test tube, creaming in nanoemulsions was assessed. The cream layer volume was measured using the graduations on the test tube after the recommended storage period, and the creaming % was calculated using:

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

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) had its radical scavenging activity on the MO and NMO determined by adding 2 ml of sample to 2 ml of 0.1 mM DPPH dissolved in 95% methanol. The liquid was stirred rapidly for 10 seconds and then kept in a dark area for 30 minutes. At a wavelength of 517 nm, the sample's absorbance was measured, and the DPPH solution without the sample acted as a blank. The following formula was used to compute the percentage of DPPH scavenging activity according to Duan et al. (2016);

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

where, A sample = absorbance of the DPPH solution after the reaction with the sample; Ablank = absorbance of the DPPH solution without sample.

Manufacture of functional ice cream with MO nanoemulsion

The ice cream was manufactured with nanoemulsion for the suitable delivery of the MO rich-omega 3 using nanoemulsion, a dairy product, as the ice cream was prepared.

Buffalo's milk, skim milk powder, sugar, and Lacta 9050 as a stabilizer was used to prepare a plain ice milk mix with a composition of 10.5, 10.0, 14.0, and 0.10% for fat, milk solids non-fat, sugar, and stabilizer, respectively. The mixture was divided into three equal parts, the first portion without MO to create a control (C). Free MO and MO nanoemulsions were added at a rate of 3 and 6%, respectively, by replacement to keep the total solids to create T1 and T2, respectively. After preheating at 65 °C and mixing all the ingredients, the mixture was homogenized using a laboratory homogenizer ((EURO TURRAXT 20b, IKA Lobo Technik 27000 min G1), pasteurized at 81EC and cooled, then aged overnight at 5±1 °C. Just before freezing in a batch freezer (Staff Ice System, BTM 10, Rimini Italy), 0.5 % vanilla was added to the aged ice cream mixture. Overrun was calculated for all formulated ice cream using the weight-volume method described by Adapa et al. (2000). The resultant ice milk was poured into plastic cups, covered, and hardened in a deep freezer at -20 °C for 24 hrs before analysis. Three replicates were done for each batch.

Ice cream mixture properties

The pH value of all ice cream mixtures was measured using a laboratory pH meter with a glass electrode (HANNA, Instrument, Portugal). The acidity content was determined by adding 0.1N sodium hydroxide to the phenolphthalein endpoint, as described by Arbuckle (1986). The whipping abilities of the ice cream mixtures were determined using a mixer with 2.6 cm blades Heidolph No. 50 111, Type RZRI, Germany) according to the method described by Baer et al.(1999).

Ice cream properties

The melting properties of the ice cream were determined according to Naeem et al.(2019).

Sensory evaluation

The ice milk samples were evaluated for appearance, melting quality, body and texture, and flavor by a regular taste panel of 21 staff members from The Food Science and technology department, Home Economic Al-Azhar University, Egypt. The ice cream was sensorial evaluated using the nine-point hedonic scale, ranging from extremely (9 points) through like or dislike (5 points) to dislike extremely (1 point) according to the method of Naeem et al.(2019).

Statistical analysis

Analysis of variance (ANOVA) and Duncan's multiple comparison procedure were used to compare the means using software SPSS version 15.0 (SPSS Inc. Chicago, Illinois). A probability of $p<0.05$ was used to establish statistical significance.For the ice cream samples, extraction of phenolic compounds and antioxidant activity were carried out according to Li *et al.*[33] with some modifications as follows: addition of 20 mL of the solvent (15 ml 1N HCl and 85 ml ethanol 95%) to 10 g of ice cream in 50-mL brown bottles and shaking for 90 min at 30°C in a rotary shaker (Julabo D-7633 Labortechmik, GMBIT, Jeelback / wast Germany) set at 200 rpm. Then, the mixture was centrifuged at 2500 g (ICE PR-7000 centrifuge, International Equipment Company) for 45 min at 5°C. The supernatant fluids were analyzed for TPC and DPPH scavenging activity as described earlier.

RESULTS AND DISCUSSION

Chemical properties of Moringa oleifera Seeds and oil:

Table 1 shows the chemical composition of the Moringa oleifera seed. Moringa seed contained 44.33 ± 1.09% oil, whereas the total proteins, total carbohydrates, and ash were 24.35 ± 0.15, 17.85 ± 0.72, and 5.57 ± 0.23%, respectively. Protein levels in Moringa oleifera seeds ranged from 7.8 to 25 g/100

g (Özcan, 2020). According to Abdulkarim et al. (2005) research, this product comprised 16.5–17.8% carbohydrates. The crude fibre was determined to have a value that ranged from 6.60 to 9% by Anwar et al. (2006).

Table 1. Chemical composition of M. oleifera seed

Composition (%)	Obtained values
Moisture content	7.20 ± 0.75
Protein	25.35 ± 0.15
Oils	44.33 ± 1.09
Fibre	7.95 ± 0.25
Ash	5.57 ± 0.23
Total carbohydrate	17.85 ± 0.72

Table 2 shows the results of the extracted oil's different physical and chemical analyses. The value for density is 0.913 ± 0.01 mg/cm3, iodine 67.45 ±0.11, and refractive index 1.455 ± 0.003. The acid value (AV), which measures overall acidity, was discovered to be 0.02 mg KOH/g oil. This value was practically on par with other Moringa oils studied in other countries. An analysis's low oil AV suggests the oil is well resistant to hydrolysis (Manzoor et al. 2007). One of the essential assays for determining oxidative rancidity in oils and fats is the peroxide value (PV), which assesses the quantities of peroxides and hydroperoxide produced during the early stages of lipid oxidation. In fresh oils, the PV should typically be less than 10 meq O2/kg oil since any rise in this number (>10) is unstable and quickly turns rancid (having an unpleasant odor) (Pearson, 1976). The PV of M. oleifera oil in this investigation was 0.61 ± 0.03 meq peroxide/kg; the result from Saudi Arabia was closest 0.4 meq/kg oil (Manzoor et al. 2007). However, the reduced PV of Moringa oil was a promising sign that some products were free of oxidative rancidity. This finding is consistent with that discovered by Anwar & Bhanger , (2003) about the induction duration (Rancimat; 9.30 0.15 h /L, 120 °C), a characteristic of the oxidative stability of the oil (Anwar & Bhanger, 2003). As compared to common vegetable oils, M. oleifera oil showed greater IP and oxidative stability (Anwar & Bhanger, 2003), which was attributed to its much higher concentration of monoenoic fatty acids, notably C18:1, which is less susceptible to oxidation than polyenoics (Anwar & Bhanger, 2003). Moreover, M. oleifera oil's great resistance to oxidation might be attributed to its high concentrations of R-, γ-, and δ-tocopherols. High stability of seed oil from the Moringaceae family was also found by Somali et al. (1) and Sengupta et al. (2). The iodine value (IV) assesses the stability of oil in industrial applications and indicates the degree of oil unsaturation (Xu et al., 2007). M. oleifera oil had an IV of 67.45 ± 0.11 mg I2/100 g. In addition, the oil's low IV represented its qualities, including its stronger oxidation resistance, longer shelf life, and higher quality. The oil from M. oleifera has a saponification number (SpN) of 179.8 ± 0.45 mg KOH /g oil and an unsaponifiable matter (USM) matter of 0.87 ± 0.14%, respectively. The lower SpN of Moringa oil suggests that it contains a significant amount of triacylglycerols with low molecular weight. Yet, the physicochemical characteristics of M. oleifera closely match those of data previously published in comparable Moringa seed oil from other countries (Manzoor et al. 2007). For instance, the values of AV, IV, PV, SN, and SM (Table 2) in Egyptian M. oleifera oil were very comparable to those found in M. oleifera oil from Pakistan, India, and Saudi Arabia (0.34%, 69.6 mg I/100 g oil, 0.40 meq kg-1 of oil, 179 mg of KOH/g oil, and 0.78%, respectively) (Anwar & Bhanger, 2003). Yet, several physico-chemical characteristics of the oil significantly differed from those found in oil from other Moringa species. Generally, the differences in genotypes, growing conditions, regional geological conditions, ripening stage, seed harvesting time, and extraction techniques between Egyptian M. Oleiefera and the same M. oleifera grew in other countries may acct for the variability in oil yield and physical-chemical characteristics (Rahaman et al. 2009).

Table 2. Characterization physicochemical of M. oleifera seed oil.

Properties	evel

Refractive index (40 °C)	**1.455 ± 0.003**
density (25 °C) (g/cm$^{3)}$	**0.913 ± 0.01**
Iodine value (g I_2/100 g oil)	**67.45 ± 0.11**
Acid value (mg KOH/g oil)	**0.05 ± 0.01**
Saponification value (mg KOH/g oil)	**179.8 ± 0.45**
Peroxide value (meq O_2/kg oil)	**0.61 ± 0.03**
Unsaponifiable matter (%)	**0.87 ± 0.14**
Oil stability index (h)	**9.30 ± 0.15**
Vit. E Conc. (µg/g)	**104.19**
Colour parameters	
Red	**1.6 ± 0.1**
Yellow	**30.00 ± 0.5**

The oil had a better colour measurement than M. peregrina oil (1.6 ± 0.1 R & 30.00 ± 0.5 Y) and was comparable to M. oleifera oil reported from Kenya. Vegetable oils' colour intensity is primarily determined by the presence of different pigments, such as chlorophyll, which are efficiently eliminated during the degumming, refining, and bleaching stages of oil manufacturing. Vegetable oils with low colour index values are more desirable for cooking and household uses.

Table 3. Fatty acid composition of Moringa oleifera oil

N	Fatty acids	Relative concentration (%)
1	**Myristic acid (C14:0)**	0.31
2	**Palmitic acid (C16:0)**	15.34
3	**Palmitoleic acid (C16:1)**	0.51
4	**Stearic acid (C18:0)**	4.64
5	**Oleic acid (C18:1*cis*)**	36.63
6	**Oleic acid (C18:1*trans*)**	2.81
7	**Linoleic acid (C18:2 *cis*)**	32.65
8	**Linoleic acid (C18:2 *trans*)**	0.36
9	**γ- Linolenic acid (C18:3n6)**	3.17
10	**Linolenic acid (C18:3n3)**	0.59
11	**Arachidic acid (C20:0)**	2.03
12	**Cis-11- Eicosenoic acid (C20.1)**	0.96

The fatty acid (FA) composition of M. oleifera oil is shown in Table 3. Total saturates, or palmitic (C16:0), stearic (C18:0), and arachidic (C20:0) acids, formed 22.01% of the oil, with C16:0 (15.85%) serving as the predominate acid. Up to 76.21% of the oil comprised monounsaturated fatty acids. The most abundant fatty acid was oleic (C18:1ω-9), which made up 39.44% of all fatty acids. Linolenic acid (C18:3ω-3) was present in 0.59% less than C18 polyunsaturated linoleic fatty acid (C18:2ω-6). The content of the three primary fatty acids C18:1, C18:0, and C16:0 in the oil examined in the current investigation was very similar to that found in the M. oleifera oil native to Kenya, as reported by Tsaknis et al. (1999). C20:0 2.03% and C20:1 (0.96%) in the oil slightly differed from what was stated in the literature (Tsaknis et al., 1999). Nevertheless, the amounts of C18:1 and C16:0 in the Pakistani-native M. oleifera oil differed considerably from those found in the Saudi Arabian seed oil from M. peregrina (Tsaknis et al., 1998). In C18:1, the oil was up 6%, while in C16:0, it was down 2.4%. Gadoleic acid (C20:1) in the oil from M. peregrina was in line with expectations, and C16:1 was also found in the current analysis. The C18:1 and C18:0 concentrations of the M. oleifera oil under investigation were relatively close to those of olive oil (Sonntag, 1979), but other component fatty acids differed. This projected oil seed crop's fatty acid content matched that reported by Ferrao & Ferrao

(1970). relatively well (Ferrao & Ferrao, 1970). M. oleifera L. has a comparable fatty acid composition, according to Sengupta and Gupta (1970). According to the oil's current fatty acid composition, it belongs to the class of high-oleic oils and has a high proportion of monounsaturated to saturated fatty acids.

Effect of concentrations of WPI and moringa oil on physical properties of nanoemulsion

Table 4. Droplet size, polydisperisty index, and zeta potential of nanoemulsio moringa oil

samples	Droplet size	PDI	Zeta potential
5WPI-MO 5%	182 ± 15.76	0.351	-10.3 ± 3.62
5WPI- MO10%	288.6 ± 18.35	0.366	-17.0 ± 4.37
10 WPI-MO 5%	117.7 ± 8.96	0.247	-20.2 ±4.81
10 WPI-MO10%	43.65 ± 3.5	0.268	-30.3 ± 5.89

Effect of concentration of WPI and oil concentration on physicochemical properties of nanoemulsion

Due to the amphiphilic structure of whey protein isolate, which contains both hydrophilic and hydrophobic groups in a single molecule, whey proteins are considered essential food emulsifiers (Christiansen, et al., 2004; Sarkar, et al., 2009; Hamed, et al., 2019). The hydrophobic component of whey protein penetrates the oil phase and bonds the protein molecules to the surfaces of the oil droplets. In contrast, by combining steric and electrostatic repulsion, its hydrophilic component protrudes into the continuous aqueous phase minimizing droplet agglomeration or aggregation (Hamed, et al., 2019). This emulsion is created using ultrasound, which generates mechanical vibration and cavitation for the production of small droplets (Jemaa et al., 2019); when WPI is used as a surfactant, the WPI molecules form a layer to achieve the equilibrium of interfacial tension, which improves the stability protection of the internal phase (Güell et al., 2017). Particle size, polydispersity index (PDI), and zeta potential (surface charge) were measured as indicators of the influence of concentrations of whey protein isolate and M. Oleifera oil on the stability of the generated nanoemulsions (Hamed et al., 2019). Table 4 shows the droplet size, polydispersity index, and surface charge of nanoemulsions prepared from different concentrations of 5 and 10 % WPI or moringa oil. The droplet size of the nanoemulsion was decreased from 288.6 ± 18.35 to 43.65 ± 3.5 with the increase in WPI from 5% to 10%. This result agrees with Hamed et al. (2019), was found that particle size decreased with increased WPI from 1% to 10%. However, the droplet size of the nanoemulsion increased with increasing oil concentration, whether WPI concentrations were 5% or 10%. While the globular proteins in WPI created strong, cohesive, and viscoelastic films surrounding droplets, they allowed for the creation of microscopic nanoemulsion droplets resistant to droplet recoalescence and aggregation (Lam & Nickerson, 2013).

For the practical uses of nanoemulsions, total particle size distribution (polydispersity index) is essential for its stability (Qian & McClements., 2011). from the results, the PDI decreased with increasing WPI but increased with increasing moringa oil. The PDI lowest values were 0.247 and 0.268, with a 10% WPI, while moringa oil was 5 and 10%, respectively. The term zeta potential in colloidal systems describes the electro-kinetic potential (Mills, 1993). This potential quantifies the difference between the continuous liquid phase's dispersion medium and the stationary layer linked to the dispersed particle in these colloidal systems (Silva et al., 2012). The zeta potential value may be connected to the stability of colloidal dispersions based on the degree of repulsion between two phases and similarly charged particles (negative or positive). Table 4 shows the effect of WPI concentration on the zeta potential of the nanoemulsions created in this study. Table 4 showed that a 10% WPI concentration resulted in a high zeta potential of 30.3 5.89 mV. According to Preetz et al., 2010), a value of 30 mV (positive or negative) might be used to distinguish low-charged surfaces from highly charged ones. Moreover, (ASTM 1985) determined that zeta potentials more significant than 30 mV imply stability. An insufficient emulsifier at the 5% concentration may have caused droplets to

recoalesce and flocculate via bridging, leading to bigger droplets. The droplets may have been too tiny to fully absorb the emulsifier (Qian & McClements, 2011). Using extra emulsifiers at a concentration of 10% may have facilitated the quick and thorough coverage of newly formed droplet surfaces (Qian & McClements, 2011). Additionally, because the electrostatic repulsion between the droplets would be amplified due to the high droplet surface charge at high emulsifier concentration, the droplets would not aggregate.

Further stability studies, such as identifying destabilization processes such as creaming, sedimentation, flocculation, and coalescence, may validate the nanoemulsion system's stability potential.

Creaming index

Improved droplet packing that constrained the relative motion of emulsion droplets made the droplets resistant to creaming and coalescence. (Ye & Singh, 2006). The results show that when the WPI was 10%, the creaming separation from the emulsion or its aggregation was weak. It began to appear after three days, especially when it was 5% Moringa oil, but the rate of cream separation was evident with 5% WPI and 10% Moringa oil; it reached 10 % creaming separated. Tiny droplets are often stable against gravitational separation (creaming and sedimentation) because of their continual Brownian motion. (Kentish et al., 2008). Whey protein emulsion stability is often increased by adding co-surfactants/emulsifiers, stabilisers, and texture modifiers. (McClements & Rao, 2011).

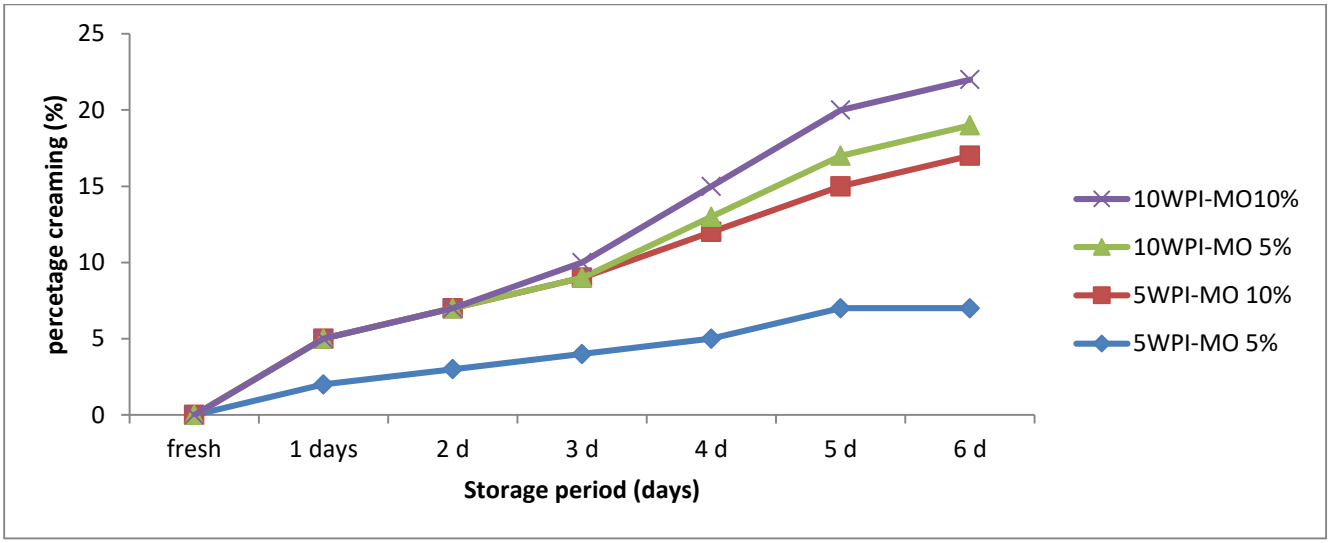


Figure1. Effect of WPI on creaming index moringa oil nanoemulsions

Ice cream manufacture with MO-nanoemulsion

Ice cream is a multiphase system that includes ice crystals, air bubbles, and fat globules in a viscous frozen matrix phase. (Eisner et al., 2005). Typical ice cream contains 10% to 16% fat. (Goff, 1997). Based on the findings of the nanoemulsion trials for ice cream manufacture, the best ratio of WPI as an emulsifier and MO addition (10%WPI-10% MO) was selected. Because more consistent findings were found, the stability of the nanoemulsion formulations based on gravity separation data at +4°C was addressed. The stability values of nanoemulsions containing 10% WPI were comparable. They were unstable during storage due to their poor viscosity and consistency factors. This investigation generated samples with the same fat concentration as ice cream produced with nanoemulsion, free MO, and ordinary fat content using traditional procedures to compare them. The control samples were manufactured after the ice cream recipes, which were made using nanoemulsion, were developed.

Vanilla Flavoured ice cream physicochemical properties:

Nanoemulsions are considered one of the most effective ways to enhance nonpolar active chemicals' solubility and bioavailability. To create innovative food products, the food industry hopes to employ these emulsions systems to include lipophilic functional substances. Table * shows the plain vanilla ice cream mix's chemical composition and antioxidant activity as the control and ice cream enriched with free MO (T1) or MO nanoemulsion (T2).

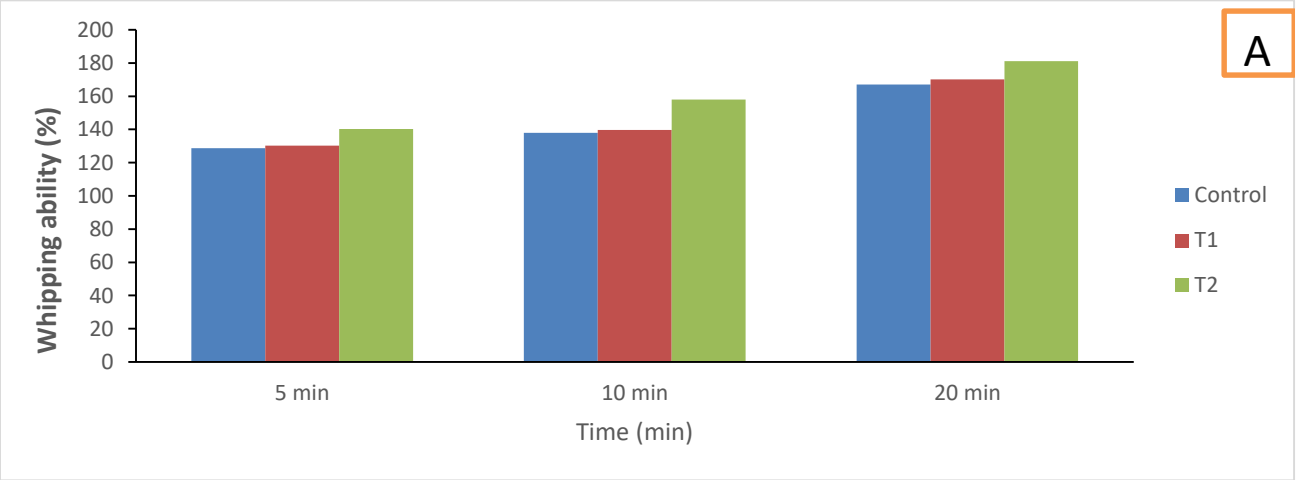
Table 5. Effect of free Moringa oil, and MO nanoemulsion on physico-chemical of vanilla ice cream

Parameters	Control	T1	T2
Total solids (%)	34.35 ± 1.15	34.45 ± 1.25	34.30 ± 1.19
Total Protein (%)	7.20 ± 0.10	7.10 ± 0.10	8.60 ± 0.15
Fat (%)	10.50 ± 0.10	10.50 ± 0.30	10.30 ± 0.20
Ash (%)	1.15 ± 0.05	1.18 ± 0.07	1.14 ± 0.03
pH	6.38 ± 0.02	6.39 ± 0.02	6.41 ± 0.02
Acidity (%)	0.35 ± 0.03	0.34 ± 0.04	0.35 ± 0.02
Antioxidant Activity (%)	32.15 ± 2.54	41.29 ± 1.85	59.75 ± 2.95
Overrun %	60 ±3.25	62.93 ± 5.21	75.10 ± 4.15

The pH of the vanilla-flavoured ice cream mixture, total solids, fat, and ash content for all treatments, which are control, T1, and T2. The total solids of all ice cream treatments were 34.35 ±1.15%. The total proteins of plain flavoured ice cream (control) and ice cream supplemented with 3% free moringa oil (T1) were 7.20 ±0.10%, increased to 8.60 ± 0.15% for ice cream fortified with MO nanoemulsion, due to adding 6 g of powdered MO nanoemulsion at a concentration of 3g MO and 3 g WPI led to a significant increase (p0.05) in total proteins compared to the control and T1 mixes. These results agree with those reported by Soliman and Shehata in 2021, who utilized curcumin whey protein isolate conjugation. The DPPH radical scavenging activity of the ice cream mix fortified by MO nanoemulsion was 59.75 ±2.95%, followed by that of the ice cream fortified with free MO (41.29± 1.85%), and the control mix (32.15 ± 2.54%). Nadeem & Ullah (2016) noticed that the antioxidant activity was improved in ice cream fortified with interesterified M. oleifera oil.

Overrun of Ice Cream

Since it impacts most of the ice cream product's qualitative characteristics, overrun is an important physical property of ice cream. Furthermore, it reliably predicts ice cream's aeration process and network creation. Additionally, it is linked to ice cream's flavour, taste, and thermal characteristics (Soukoulis et al., 2010). Increasing overrun values produced softer ice cream resulting in fewer ice crystals and air cell bubbles (Sofjan & Hartel, 2004). Compared to traditional fat-containing formulations,



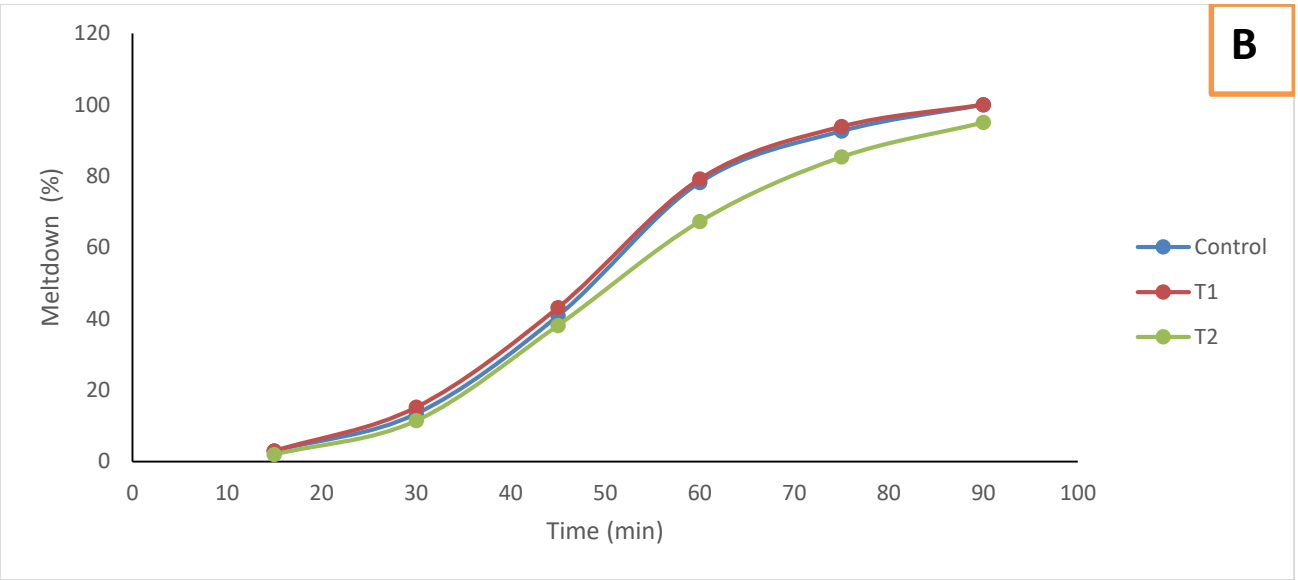


Figure 2. a) **Whipping ability (%) B) meltdawon of plain vanilla ice cream and fortified with free MO or MO nanoemulsion.**

ice cream made with MO nanoemulsion showed greater overrun values. This demonstrated that nanoemulsion increased air content and network creation in the ice cream formulations compared to standard fat ice cream due to maybe whey protein isolate.

Whey protein isolates enhance their valuable characteristics. As shown in Fig. 2a, vanilla ice cream mixes containing free MO or MO nanoemulsion have a higher volume with more extraordinary whipping times. Compared to the control and T1 mixes, the T2 mixture enhanced with MO nanoemulsion demonstrated the most vital whipping ability after 5 minutes and continued at 10 and 20 minutes ($p > 0.05$). At 5, 10, and 20 minutes, there was no discernible difference between the control mix and T1 supplemented with free Mo ($p>0.05$). But in T2 mix improve the whipping ability due to the Mo nanoemulsion emulsifier (WPI). By boosting the binding in the air-cell membrane, Soliman and Shehata (2021) observed that adding WPI-MD conjugate may have increased the air incorporation inside the frozen milk matrix.

Meltdown is the important desired parameters of ice cream quality is good shape retention and slow melting38. The meltdown of vanilla ice cream (g/100 g) formulated with free MO, or Mo nanoemulsion, is shown in Fig. 2b. After 15 min, the addition of free MO had no significant effect ($p>0.05$) on the amount of melted ice cream compared with the control. However, the melted ice cream was the lowest when added MO nanoemulsion was ($p>0.05$) compared to other treatments and followed the same pattern at 30, 45, 60 and 90 min ($p<0.05$) compared with control and T1 mixes.

Sensory Evaluation

The sensory quality of ice cream treatments is shown in Figure 3. Particularly when utilizing MO nanoemulsion, MO improved the vanilla ice cream's sensory quality scores and acceptance compared to the control. The samples' appearance characteristics changed noticeably, and the MO nanoemulsion significantly improved the colour of ice milk compared to the control. However, ice cream reinforced with free MO received the lowest taste rating. The Paneleste ice cream fortified with MO nanoemulsion received a high assessment score and is more palatable.

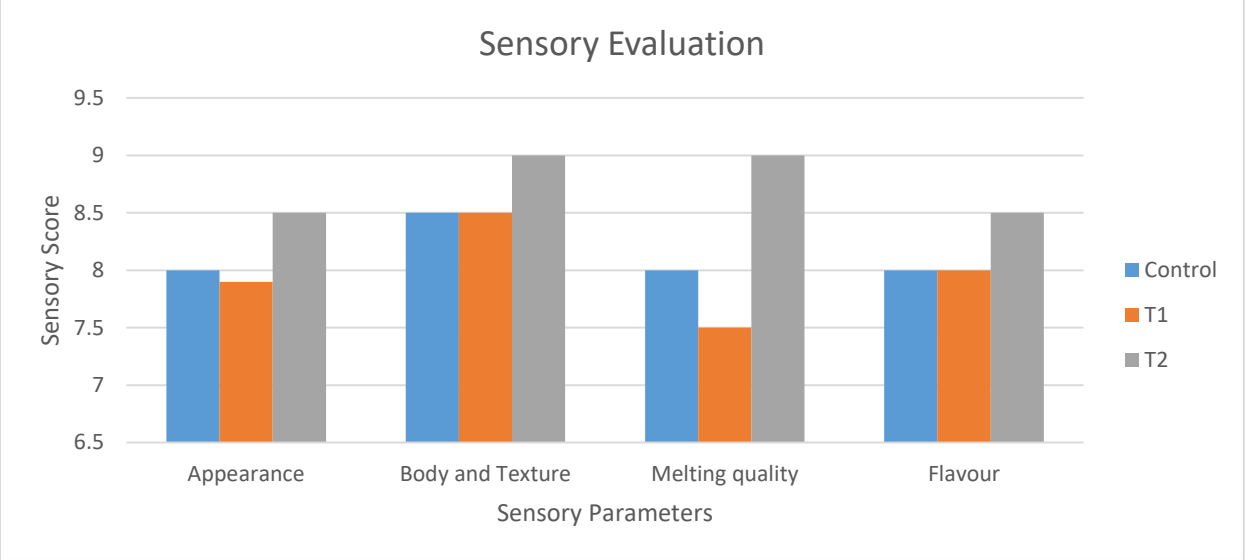


Figure 3. Sensory evaluation of Plain Vanilla ice cream and fortified with free MO or Mo nanoemulsion.

Conclusion

The present work attempted to produce functional ice cream fortified with moringa seed oil-based nanoemulsion. The omega-3-rich oil-in-water nanoemulsions were prepared by ultrasonic homogenization. Such emulsification procedures demonstrated good nanoemulsion characteristics regarding the acceptable small droplet size, unimodal polydispersity index and good zeta potential. The prepared ice cream fortified with MO-based nanoemulsions showed good physicochemical characteristics and acceptable sensory attributes.

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