

## Physicochemical properties of functional yoghurt fortified with microencapsulated moringa and black cumin oils

A. F. Elshiekh<sup>1</sup>, M. A. Omar<sup>1</sup>, A. I. Mansour<sup>2</sup>, M. A. Ahmed<sup>2</sup>, D. M. Mohammed<sup>3</sup>, and T. M. El-Messery<sup>4</sup>

<sup>1</sup> Dairy Department, Faculty of Agriculture, Al-Azhar University, (Cairo)

<sup>2</sup> Dairy Department, Faculty of Agriculture, Al-Azhar University (Assiut)

<sup>3</sup> Nutrition and Food Sciences Department, National Research Centre, Cairo, 12622, Egypt

<sup>4</sup> Dairy Department, National Research Centre, Cairo, 12622, Egypt

\* Corresponding author E-mail: (A. Elshiekh)

### ABSTRACT:

Microcapsules of *Moringa oleifera* (moringa) and *Nigella sativa* (Black Cumin oils) were formed by using maltodextrin (MD), whey protein concentrate and gum Arabic (GA) as carrier agents using freeze drier. Microcapsules were added to yoghurt. The characteristics of either the microcapsules or the functional fortified yoghurt were studied. The characterization of micro emulsions with different concentrations of moringa and *Nigella sativa* (Black Cumin oils) revealed stable emulsions with negative zeta potentials (-31.4 to -41.8 mV for moringa oil and -32.1 to -37.0 mV for black cumin oil detected). Particle sizes ranged from 442.5 to 550.7 nm for *Moringa oleifera* oil and 312.5 to 642.1 nm for *Nigella sativa* (Black Cumin oil). Encapsulation efficiency was highest at 5% oil concentration but decreased at higher concentrations. Adding encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) to yoghurt affected its viscosity, pH, and acidity. The fortified yoghurt with the oils showed microbiological properties and absence of harmful bacteria. Sensory evaluation indicated that an optimal concentration of 4% for both oils enhanced flavor and texture. Generally, the addition of encapsulated oils improved the nutritional value and potential health benefits of yoghurt.

**Keywords:** Yoghurt; Physical encapsulation; Freeze drier; *Moringa oleifera*; *Nigella sativa*.

### INTRODUCTION:

Yoghurt, a traditional fermented dairy product, has gained significant popularity worldwide due to its nutritional benefits and potential health-promoting properties (McKinley *et al.*, 2005). It is a rich source of high-quality protein, essential vitamins, and minerals, along with probiotics that support gut health (Gómez-Gallego *et al.*, 2018). In recent years, there has been a growing interest in fortifying yoghurt with bioactive compounds to enhance its functional properties and offer additional health benefits (Sharma *et al.*, 2023). One of these approaches is the incorporation of microcapsules containing oils derived from Black Cumin (*Nigella sativa*) and Moringa (*Moringa oleifera*), which are known for their potential therapeutic effects (Islam *et al.*, 2021 and Rahim *et al.*, 2022). Black Cumin oil, derived from the seeds of *Nigella sativa*, is rich in bioactive compounds such as thymoquinone, thymohydroquinone and thymol (Tiruppur Venkatachallam *et al.*, 2010). These compounds possess antioxidant, anti-inflammatory, antimicrobial and anticancer properties, making Black Cumin oil a promising candidate for functional food fortification (Rahim *et al.*, 2022). Moringa oil, extracted from the seeds of *Moringa oleifera*, is highly nutritious and

contains a range of bioactive compounds, including vitamins, minerals, antioxidants, and phytochemicals (Falowo *et al.*, 2018). It has been associated with various health benefits, including anti-inflammatory, antimicrobial and hepatoprotective effects (Kou *et al.*, 2018). The encapsulation of Black Cumin and Moringa oils in microcapsules offers several advantages for their incorporation into yoghurt (Rout *et al.*, 2022 and Calderón-Oliver & Ponce-Alquicira (2022). Microencapsulation protects the oils from oxidation, light, and heat, ensuring their stability, preserving their bioactive properties during storage, and processing (Dias *et al.*, 2015). Additionally, microcapsules provide controlled release of the encapsulated oils, enabling their gradual release during digestion and maximizing their bioavailability and health benefits (Liu *et al.*, 2022). It is crucial to highlight that although scientific studies have provided evidence for the potential health advantages associated with the individual constituents, such as Black Cumin oil, Moringa oil, and probiotics, further research are required to specifically examine the collective impact of these elements in fortified yoghurt. Additionally, individual responses to fortified yoghurt may vary, and it is always advisable to consult with a professional healthcare institute or nutritionist before making significant changes to the

human diet. The fortification of yoghurt with microcapsules of *Moringa oleifera* and *Nigella sativa* (Black Cumin) oils opens new opportunities to develop functional yoghurt with enhanced nutritional and therapeutic properties (Nazari *et al.*, 2023 and Ali *et al.*, 2022). The combined effects of the bioactive compounds present in these oils and the probiotics in yoghurt may exert synergistic health effects, such as improved gastrointestinal health, enhanced immune function, and potential disease prevention (Tomas *et al.*, 2022). Several studies have investigated the incorporation of microcapsules containing *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) into different food matrices, highlighting their potential applications in functional foods. For instance, research by Ahmed *et al.* (2019) demonstrated the successful encapsulation of Black Cumin oil in microcapsules and its incorporation into yoghurt, resulting in improved antioxidant activity and sensory characteristics. Moreover, a study by Gomes *et al.* (2023) investigated the encapsulation of *Moringa oleifera* oil in alginate-based microcapsules and its incorporation into probiotic-enriched yoghurt, revealing enhanced functional properties and potential health benefits. The aim of this study, fortification of yoghurt with microcapsules containing *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) represents a promising approach to develop functional yoghurt with enhanced nutritional and therapeutic properties. Further research is needed to explore the optimal encapsulation techniques, dosage levels and long-term effects of these fortified yoghurts on human health, paving the way for the development of innovative functional foods.

## MATERIALS AND METHODS

### Materials

*Moringa oleifera* and *Nigella sativa* (Black Cumin oils) were acquired from the oil's extraction unit at the National Research Centre in Egypt. Low heat skimmed milk powder with a composition of 34% protein, 0.5% fat, and 4% moisture was obtained from The Nile Commercial CO. in Cairo, Egypt. The yoghurt starter culture (YC-X11), which contained *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, was sourced from the Chr. Hansen laboratory in Denmark. Maltodextrin (MD), Whey protein concentrate (WPC), and Arabic gum (AG) were purchased from Aromsa Co. in Turkey. Hexane was obtained from Sigma-Aldrich Co. in the USA.

### Preparation of micro emulsions with different oil concentrations

To prepare the micro emulsions with varying oil concentrations, a solution of MD (10% w/v) and AG (10% w/v) was created by dissolving them in distilled water at temperatures of 50-60°C for a duration of 30 min. The WPC was dissolved in distilled water at temperatures of 60-80°C for 30 min to activate its active components. Based on a study by Chung *et al.* (2011), a 4:1 ratio of MD to WPC was selected for the preparation of the oil's micro emulsion formulation. The MD and WPC solutions were mixed at this ratio and stirred for 1 h. Different concentrations of *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) (5, 10, 15 and 20% w/v) were added to the MD: WPC solution and the mixtures were homogenized using an Ultra-Turrax homogenizer (18000 rpm/20 min). The resulting mixtures were sonicated for 20 min on an icebox. A solution of AG (0.5% in total) was added to the micro emulsion to enhance its stability, and the mixture was homogenized again.

### Dehydration of micro emulsions by freeze-drying:

The micro emulsions were freeze-dried to form microcapsules from *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) by freezing them at -20°C and then drying them using a lyophilizer. The dried product was collected after 48 hours of freeze-drying and stored in an airtight container at -20°C for further analysis according to Abdelwahed *et al.* (2006).

### Characterization of micro emulsions

#### Micro emulsion stability

The stability of the micro emulsion was determined by measuring the separation of serum from the emulsion. The emulsion was stored in a cylinder for different time intervals, and the height of the separated serum was measured to calculate the percentage of separation by utilizing the equation (1) followed:  $\% \text{ Separation} = \frac{H_1}{H_0} \times 100$

Where: H1 is the height of upper phase; H0 represents the initial height of emulsion.

#### Measurement of droplet size and zeta potential of micro emulsions

The particle size distribution and zeta potential of the emulsion droplets were measured using a Malvern Zetasizer Nano Z. The samples were diluted with distilled water

before the measurements (Ramadan *et al.*, 2021).

### Transmission electron microscopy (TEM)

The morphology of the Nano emulsions was examined using transmission electron microscopy. Prior to imaging, the samples were coated with a thin layer of gold to facilitate electron microscopy.

### Characterization of freeze-dried micro-emulsions

#### Encapsulation efficiency (EE)

The encapsulation efficiency was determined by extracting the free oil from the dried micro emulsion powder using hexane (Abdel-Razek *et al.*, 2022). The remaining extract represented the encapsulated amount of oil, and the encapsulation efficiency was calculated from the following equation (2):

$$EE\% = \frac{TPC - FPC}{TPC} \times 100$$

Where: TPC: Total phenolic compounds; FPC: Free phenolic compounds

### Surface morphology of freeze-dried micro-emulsions

Scanning electron microscopy (SEM) was used to visualize the surface morphology of the freeze-dried micro-emulsions.

### Yoghurt preparation

Yoghurt was prepared by pasteurizing reconstituted skim milk (12% T.S) at 85°C for 30 sec and then cooling it to 42°C. The milk was inoculated with a yoghurt culture (*S. thermophilus* and *lb. delbrueckii ssp. bulgaricus*) at a concentration of 3% (w/v) and incubated at 42°C until the pH dropped to 5.2 and 4.6. The yoghurt samples were divided into four portions: Control yoghurt (labeled C): This portion was prepared without any additives and the other portions T1, T2, T3 and T4 yoghurt: This portion contained 2, 4, 6 and 8% dried micro emulsions; respectively. All yoghurt samples were stored at 4°C for detected of 14 days of cold storage. This experiment was replicated three times to ensure the reliability and consistency of the results.

### Yoghurt's physical and chemical properties

The acidity and pH of the yoghurts were measured immediately after manufacture at 25C using a pH meter (pH 211, HANNA Instruments, Leighton Buzzard, UK). The titration method was used to determine the acidity % as lactic acid (Mohamed *et al.*, 2017).

The apparent viscosity of the yoghurts was determined using a dynamic viscometer (Brookfield Model-LV; Brookfield Engineering Laboratory, Stoughton, USA) was used at a speed of 100 rpm (El-Said *et al.*, 2018).

### Microbiological analysis

#### Coliform bacterial count

Coliform bacterial counts were determined using Violet Red Bile Agar (VRBA) followed by incubation for 1-2 days at 37°C according to APHA (2000).

#### Moulds and yeasts count

Moulds and yeasts counts were determined using the pour plate method by using Malt Extract Agar (MEA) followed by incubation for 3-5 days at 25°C according to APHA (2000).

### Sensory evaluation

Sensory evaluation of the yoghurts was performed using a 10-point hedonic scale to assess appearance, flavor, and texture. The evaluations were conducted when the yoghurt was fresh and after 7 & 15 days of storage (Mohamed *et al.*, 2017).

### Statistical analysis

The experimental results were presented as mean values  $\pm$  standard deviation, and each experiment was repeated at least three times. Statistical analysis of the data was conducted using Minitab 18 software, developed by Minitab Ltd. in Coventry, UK. ANOVA and Duncan's multiple range tests were applied to examine differences at a 5% significance level ( $P < 0.05$ )

## RESULTS AND DISCUSSION

### Characterization of the micro emulsions with different oils concentration

#### Micro emulsion stability, zeta potential and Particle size

Table 1 presents the stability of micro emulsions and the zeta potential of oil droplets in micro emulsions with different concentrations of *Moringa oleifera* and *Nigella sativa* (Black Cumin oils). Micro emulsions containing 5 to 15% (w/v) of moringa oil did not show any phase separation, while those containing 20% (w/v) of moringa oil exhibited 35% phase separation after 10 days. Similarly, micro emulsions with 5 and 10% (w/v) of *Nigella sativa* (Black Cumin oil) showed no phase separation, but those with 15 and 20% (w/v) of *Nigella sativa* (Black Cumin oil) showed 23 and 65% phase separation after 10

days, respectively. These results suggest that insufficient emulsifier concentration resulted in faster droplet coalescence (Abdel-Razek et al., 2018). Zeta potential measures the charge attraction or repulsion between particles and plays a role in preventing droplet coalescence and ensuring emulsion stability (Premi and Sharma, 2017). Table 1 presents the zeta potential of nano emulsion droplets as influenced by the concentrations of *Moringa oleifera* and *Nigella sativa* (Black Cumin oils). The zeta potential of micro emulsions with different concentrations of *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) ranged from -31.4 to -41.8 mV and from -32.1 to -37.0 mV: respectively. The negatively charged carboxylate groups on the outer surface of droplets, formed by WPC and AG, resulted in a negative zeta potential under neutral pH conditions. AG always has a negative zeta potential regardless of pH due to carboxylate groups (Kaushik et al., 2016). Emulsions with zeta potentials higher than -30 mV or lower than -30 mV tend to be electrostatically stable (Premi and Sharma, 2017). Previous studies have reported that micro emulsions containing 2-8% oil exhibited negative zeta potentials ranging from -25.0 to -48.8 mV, and these emulsions showed good stability without significant effects on oil concentration (Cansell et al., 2003). The negative zeta potential increased with the dispersion of micro emulsion droplets, explaining the good emulsion stability (McClements, 2005). The zeta potential results of this study are consistent with the emulsion stability values and support previous findings regarding the behavior of WPC/AG (Weinbreck et al., 2004). Within this range of zeta potential, covalent cross-linking between WPC and AG occurs, leading to repulsion between droplets and the prevention of clotting and creaming of encapsulated droplets. The zeta potential results in Table 1 confirm the formation of the desired complex of WPC and AG on the emulsion surface. The droplet sizes of micro emulsions containing different concentrations (5-20% w/v) of *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) were measured using a Malvern Zetasizer Nano Z, and the results are presented in Table 1. High-pressure homogenization at 172.3 MPa resulted in the formation of micro emulsions with Nano sized droplets, with droplet diameters ranging from 442.5 to 550.7 nm for *Moringa oleifera* oil and from 312.5 to 642.1 nm for *Nigella sativa* (Black Cumin oil). The droplet size in the micro fluidization approach depends on the interaction chamber, operating conditions, and

emulsion composition (Villalobos-Castillejos et al., 2018). While no linear correlation was found between the concentrations of *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) and droplet size in the 5-15% (w/v) formulations, there was an increase in droplet size at 20% (w/v) concentrations of both oils.

#### **Transmission electron microscopic (TEM)**

TEM analysis was performed on single-layer emulsions to examine their microstructure, as depicted in Fig 1. Fig 1A and 1B show the morphology of MeBCO and MeMO droplets coated with AG in the primary emulsions. The TEM images revealed that the droplets had a distinct spherical shape with a well-defined interface corresponding to the AG layer. The droplet sizes observed through TEM were consistent with those measured using dynamic light scattering. The size distribution of the droplets was found to be heterogeneous, indicating the presence of certain aggregates. These findings are consistent with the reported values of the Polydispersity Index (PDI) shown in Table 1. Overall, these results confirm that the microstructures of the emulsions varied depending on the composition of the layers surrounding the droplets.

#### **Characterization of freeze-dried micro emulsions**

##### ***The % encapsulation efficiency (EE) of freeze-dried micro emulsions***

Table 2 presents the encapsulation efficiency (EE) of freeze-dried microemulsions with different concentrations (5, 10, 15 and 20% w/v) of *Moringa oleifera* and *Nigella sativa* (Black Cumin oils). The EE of the encapsulated oils ranged from 80.3 to 50% for *Moringa oleifera* oil and from 82.4 to 50.4% for *Nigella sativa* (Black Cumin oil). Higher EE values were found in formulations with 5% (w/v) of oils. However, when the oil concentration exceeded 10% (w/v), the EE was affected by the oil content. Significantly, different EE values were observed between samples with 10, 15 and 20% (w/v) of oils. Fig 2 displays the particle structure images of *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) encapsulated using freeze-drying techniques, as observed through scanning electron microscopy (SEM). The SEM images revealed that the freeze-dried powder had sharp edges, a broken glass-like surface, and a brittle texture, which resulted from the lyophilization process and the extended drying period (Khazaei et al., 2014). Sahin-Nadeem et al. (2013) mentioned that the outer topography of

freeze-dried particles exhibited a spherical shape with shallow dents of shrinkage and without cracks or pores, attributed to the protein in the wall material. The absence of pores or cracks on the particle surface is crucial for preventing the inward diffusion of oxygen and thereby providing better protection for the encapsulated extract. Additionally, this feature significantly enhances the oxidative stability of freeze-dried microcapsules, partly due to the lower overall surface area and lower surface extract content.

### Yoghurt's physical and chemical properties

According to the findings presented in Table 3, it was observed that when the milk mixed with varying concentrations of encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) was fermented using a yoghurt starter, the pH of the mixture began to decrease. The Table also provided information on the time (min) required the pH to reach the levels of 5.2 and 4.6. It was clear from these results that the time needed to reach pH 5.2 and 4.6 increased with increasing the addition of encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils). This result clearly indicated that, slowness of lactic acid increments concomitant to the addition of encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) and positively related to the ratio of additives. This finding is presumably due to some inhibitory action of the added of encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) on yoghurt starter activity, as reported by El-Sayed *et al.* (2016) and Abdel-Razek *et al.* (2018).

### The apparent viscosity

The results presented in Table 4 indicate that the addition of different concentrations of encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) to the milk, followed by fermentation with a yoghurt starter, led to a decrease in pH. The Table also includes data on the time required (min) for the pH to reach the specific levels of 5.2 and 4.6. Continuous shearing resulted in a reduction in the viscosity of the yoghurt, meaning that higher viscosity required longer shearing time. The data in the same Table showed the changes in viscosity for both the control samples (without encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) and the fortified yoghurt over the shearing time. It was observed that the encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) treatments had higher shearing times compared to the control samples, indicating that the addition of

encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) resulted in increased viscosity of the yoghurt. This is due to an increase in the wall materials like MD and WPC can bind water, leading to an increase in the viscosity of the medium. This explanation aligns with the findings of the study conducted by Quintanilha *et al.* (2021). Furthermore, the monitoring of shearing time in both the control and fortified yoghurt revealed that the viscosity of the control yoghurt significantly decreased during the first 2 min of shearing and then slightly decreased further until the end of the shearing time. In the case of the fortified yoghurt, the viscosity of the treatments containing *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) showed a significant decrease during the first 3 min of shearing and remained mostly unchanged thereafter. These results align with a study conducted by Cardines *et al.* (2018).

### pH and acidity

The changes in pH and acidity values of yoghurt samples during a 14-day storage period at 4°C were recorded and presented in Table 5. It was observed that the pH values of fortified yoghurt with encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) showed a significant difference compared to the control yoghurt when fresh. Furthermore, throughout the storage period, all yoghurt treatments exhibited noticeable changes in pH values. These findings align with a study conducted by Abdel-Razek (2019), which likely reported similar observations regarding the changes in pH values of yoghurt samples during storage. In terms of titratable acidity, the yoghurt fortified with encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) had an opposite trend for pH value throughout the storage period. This observation is consistent with a study conducted by Abdel-Razek (2019).

### Microbiological analysis

Based on the data presented in the Table 6, it was observed that the fortified yoghurt with encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) showed the absence of coliform bacteria, as well as moulds and yeasts. This absence of microorganisms may be attributed to the inhibitory effect of *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) on coliform organisms. Furthermore, both *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) are considered to have antibacterial properties against pathogenic microorganisms, which could potentially enter the bio yoghurt

either before or after processing, thereby ensuring the safety of the product for human consumption. These findings are consistent with studies conducted by Rahman *et al.* (2009) and Mohammed (2022), which deleted reported similar results regarding the absence of coliform bacteria and the antimicrobial properties of *Moringa oleifera* and *Nigella sativa* (Black Cumin oil).

#### Sensory evaluation:

The sensory evaluation was conducted on yoghurt fortified with encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) (Table 7). The results presented in the Table showed the organoleptic scores of the control yoghurt and the fortified yoghurt with different concentrations of encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils). It was observed that the encapsulated *Moringa oleifera* oil treatments received lower scores compared to the control bio yoghurt. The highest scores were obtained at 4% concentration, and the scores gradually decreased as the percentage of encapsulated *Moringa oleifera* oil increased up to 8%. The addition of *Moringa oleifera* oil appeared to enhance the flavor and consistency of the yoghurt, resulted in lower scores for flavor, body, and texture. Similar findings were reported by Amer *et al.* (2014). Regarding the fortified yoghurt with different concentrations of encapsulated *Nigella sativa* (Black Cumin oil), the treatments initially received higher scores than the control yoghurt, up to 4% concentration. However, the scores gradually decreased as the percentage of encapsulated *Nigella sativa* (Black Cumin oil) increased up to 8%. This observation is consistent with the findings reported by Okur (2021). In general, based on the obtained results, it is recommended to use encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) up to 4% to achieve a highly acceptable product with enhanced health benefits.

#### CONCLUSION

The characterization of micro emulsions containing *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) provides valuable insights into their stability, particle size, encapsulation efficiency, and other properties. To assess the stability of the emulsions, phase separation was examined, and the results indicated that emulsions with lower concentrations of oil (5-15% w/v for *Moringa oleifera* oil and 5-10% w/v for *Nigella sativa* oil deleted) exhibited stability without any signs of phase separation. However, higher concentrations led to phase

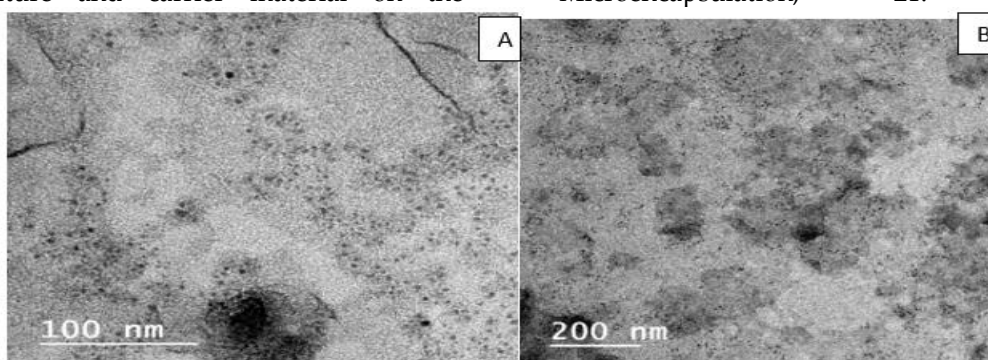
separation after a certain period, indicating that the importance of sufficient emulsifier content to maintain stability. In conclusion, the addition of encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) to yoghurt affected its pH, viscosity, microbiological properties, and sensory characteristics. The pH of the yoghurt decreased with increasing oil concentrations, while the viscosity increased. The fortified yoghurt showed an absence of coliform bacteria, molds, and yeasts, indicating potential antimicrobial properties of the oils. Sensory evaluation revealed that the organoleptic scores varied depending on the oil concentration, with lower scores observed for higher concentrations. Based on the study's findings, it is recommended to limit the supplementation of encapsulated *Moringa oleifera* and *Nigella sativa* (Black Cumin oils) to 4% in yoghurt to achieve a desirable product with enhanced health benefits. Further research is necessary to explore other quality attributes and optimize the incorporation of these oils into yoghurt.

#### REFERENCES

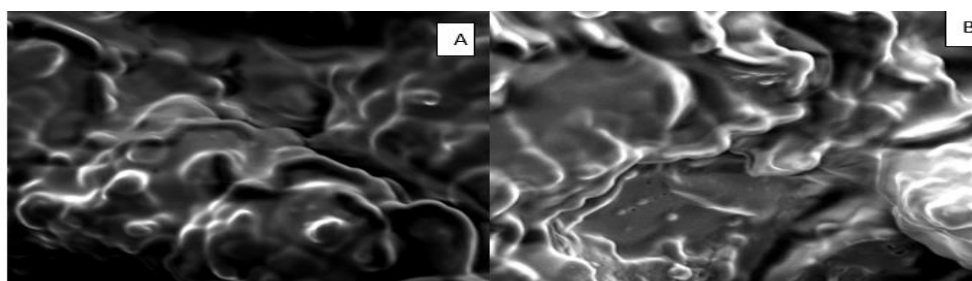
- Abdel-Razek, A.G. 2019: Non-traditional Oils Encapsulation as Novel Food Additive Enhanced Yogurt Safety Against Aflatoxins. Pakistan Journal of Biological Sciences: PJBS, 22(2): 51-58.
- Abdel-Razek, A.G., Badr, A.N., El-Messery, T.M., El-Said, M.M., Hussein, A.M.S. 2018: Micro-nano encapsulation of black seed oil ameliorate its characteristics and its mycotoxin inhibition. Bio sci. Res., 15: 2591-2601.
- Abdel-Razek, A.G., Hassanein, M.M., Ozçelik, B., Baranenko, D.A., El-Messery, T.M. 2022: Omega fatty acid-balanced oil formula and enhancing its oxidative stability by encapsulation with whey protein concentrate. Food Bioscience, 50: 101975.
- Abdelwahed, W., Degobert, G., Stainmesse, S., Fessi, H. 2006: Freeze-drying of nanoparticles: formulation, process and storage considerations. Advanced drug delivery reviews, 58(15): 1688-1713.
- Ahmed, O.A.A., Ahmed, R.R., Mahmoud, A.I. 2019: Microencapsulation of *Nigella sativa* oil and its impact on the oxidative stability and sensory characteristics of yoghurt. International Journal Dairy Technological, 72(4): 507-515.
- Ali, M.A., Kamal, M.M., Rahman, M.H., Siddiqui, M.N., Haque, M.A., Saha, K.K., Rahman, M.A. 2022: Functional dairy products as a source of bioactive peptides and probiotics: Current trends and future prospective. Journal of Food Science and Technology, 59(4): 1263-1279.

- Amer, A.E., El-Salam, B., Salem, A.S. 2014: Effect of *Moringa oleifera* leaves extract as a growth factor on viability of some encapsulated probiotic bacteria. *World Journal Dairy Food Science*, 9: 86-94.
- APHA 2000: Compendium of methods for the microbiological examination of foods. American Public Health Association.
- Calderón-Oliver, M., Ponce-Alquicira, E. 2022: The role of microencapsulation in food application. *Molecules*, 27(5): 1499.
- Cansell, M., Nacka, F., Combe, N. 2003: Marine lipid-based liposomes increase in vivo fa bioavailability. *Lipids*, 38: 551-559.
- Cardines, P.H., Baptista, A.T., Gomes, R.G., Bergamasco, R., Vieira, A.M. 2018: *Moringa oleifera* seed extracts as promising natural thickening agents for food industry: Study of the thickening action in yogurt production. *Lwt*, 97: 39-44.
- Chung, S.H., Lee, D.W., Kim, M.S., Lee, K.Y. 2011: The synthesis of silica and silica-ceria, core-shell nanoparticles in a water-in-oil (W/O) microemulsion composed of heptane and water with the binary surfactants AOT and NP-5. *Journal of colloid and interface science*, 355(1): 70-75.
- Dias, M.I., Ferreira, I.C., Barreiro, M.F. 2015: Microencapsulation of bioactive for food applications. *Food & function*, 6(4): 1035-1052.
- El-Said, M.M., El-Messery, T.M., El-Din, H.M. 2018: The encapsulation of powdered doum extract in liposomes and its application in yoghurt. *Acta Scientiarum Polonorum Technologia Alimentaria*, 17(3): 235-245.
- El-Sayed, O.F., El-Taweel, H.S., El-Shibiny, A.A., Kamal, M.M. 2016: Using moringa leaves powder in production of probiotic yoghurt. *Sinai Journal of Applied Sciences*, 5(2): 197-208.
- Falowo, A.B., Mukumbo, F.E., Idamokoro, E.M., Lorenzo, J.M., Afolayan, A.J., Muchenje, V. 2018: Multi-functional application of *Moringa oleifera* Lam. in nutrition and animal food products: A review. *Food research international*, 106: 317-334.
- Gomes, S.M., Leitão, A., Alves, A., Santos, L. 2023: Incorporation of *Moringa oleifera* Leaf Extract in Yoghurts to Mitigate Children's Malnutrition in Developing Countries. *Molecules*, 28(6): 2526.
- Gómez-Gallego, C., Gueimonde, M., Salminen, S. 2018: The role of yogurt in food-based dietary guidelines. *Nutrition reviews*, 76(1): 29-39.
- Islam, Z., Islam, S.M., Hossen, F., Mahtab-ul-Islam, K., Hasan, M.R., Karim, R. 2021: *Moringa oleifera* is a prominent source of nutrients with potential health benefits. *International Journal of Food Science*, 2021.
- Kaushik, P., Dowling, K., McKnight, S., Barrow, C.J., Adhikari, B. 2016: Microencapsulation of flaxseed oil in flaxseed protein and flaxseed gum complex coacervates. *Food research international*, 86: 1-8.
- Khazaei, K.M., Jafari, S.M., Ghorbani, M., Kakhki, A.H. 2014: Application of maltodextrin and gum Arabic in microencapsulation of saffron petal's anthocyanins and evaluating their storage stability and color. *Carbohydrate polymers*, 105: 57-62.
- Kou, X., Li, B., Olayanju, J.B., Drake, J.M., Chen, N. 2018: Nutraceutical or pharmacological potential of *Moringa oleifera* Lam. *Nutrients*, 10(3): 343.
- Liu, K., Chen, Y.Y., Pan, L.H., Li, Q.M., Luo, J.P., Zha, X.Q. 2022: Co-encapsulation systems for delivery of bioactive ingredients. *Food Research International*, 155: 111073.
- McClements, D.J. 2005: Principles, practice, and techniques. Boca Raton: Crc Press.
- Mckinley, M.C. 2005: The nutrition and health benefits of yoghurt. *International journal of dairy technology*, 58(1), 1-12.
- Mohamed, D.A., Hassanein, M.M., El-Messery, T.M., Fouad, M.T., El-Said, M.M., Fouda, K.A., Abdel-Raze, A.G. 2017: Amelioration of diabetes in a rat model through yoghurt supplemented with probiotics and olive pomace extract. *Journal Biological Science*, 17(7): 320-33.
- Mohammed, H., Mohammed, F., Velaydhanpillai, P., Berhane, N., Zemene, A. 2022: Synergistic Antibacterial Activity of Black Seed (*Nigella sativa*) and Clove (*Syzygium aromaticum*) Against Some Selected Pathogenic Bacteria. *Materials Research*, 10(1): 1-23.
- Nazari, A., Zarringhalami, S., Asghari, B. 2023: Influence of germinated black cumin (*Nigella sativa* L.) seeds extract on the physicochemical, antioxidant, antidiabetic, and sensory properties of yogurt. *Food Bioscience*, 53: 102437.
- Okur, Ö.D. 2021: Determination of antioxidant activity and total phenolic contents in yogurt added with black cumin (L.) honey. *Ovidius University Annals of Chemistry*, 32(1): 1-5.
- Premi, M., Sharma, H.K. 2017: Effect of different combinations of maltodextrin, gum arabic and whey protein concentrate on the encapsulation behavior and oxidative stability of spray-dried drumstick (*Moringa oleifera*) oil. *International journal of biological macromolecules*, 105: 1232-1240.
- Quintanilha, G.E.O., Baptista, A.T.A., Gomes, R.G., Vieira, A.M.S. 2021: Yogurt production added ultrafiltered seed extract of *Moringa oleifera* Lam. *Biocatalysis and Agricultural Biotechnology*, 37: 102159.

- Rahim, M.A., Shoukat, A., Khalid, W., Ejaz, A., Itrat, N., Majeed, I., Al-Farga, A. 2022: A narrative review on various oil extraction methods, encapsulation processes, fatty acid profiles, oxidative stability, and medicinal properties of black seed (*Nigella sativa*). *Foods*, 11(18): 2826.
- Rahman, M.M., Sheikh, M.M.I., Sharmin, S.A., Islam, M.S., Rahman, M.A., Rahman, M.M., Alam, M.F. 2009: Antibacterial activity of leaf juice and extracts of *Moringa oleifera* Lam. against some human pathogenic bacteria. *CMU Journal National Science*, 8(2): 219.
- Ramadan, M.M., El-Said, M.M., El-Messery, T.M., Mohamed, R.S. 2021: Development of flavored yoghurt fortified with microcapsules of triple omega 3-6-9 for preventing neurotoxicity induced by aluminum chloride in rats. *Journal of Food Processing and Preservation*, 45(9): 15759.
- Rout, S., Tambe, S., Deshmukh, R.K., Mali, S., Cruz, J., Srivastav, P.P., de Oliveira, M.S. 2022: Recent trends in the application of essential oils: The next generation of food preservation and food packaging. *Trends in Food Science & Technology*.
- Şahin-Nadeem, H., Dinçer, C., Torun, M., Topuz, A., Özdemir, F. 2013: Influence of inlet air temperature and carrier material on the production of instant soluble sage (*Salvia fruticosa* Miller) by spray drying. *LWT-Food Science and Technology*, 52(1): 31-38.
- Sharma, H., Ozogul, F., Bartkiene, E., Rocha, J.M., 2023: Impact of lactic acid bacteria and their metabolites on the techno-functional properties and health benefits of fermented dairy products. *Critical Reviews in Food Science and Nutrition*, 63(21): 4819-4841.
- Tiruppur Venkatachallam, S.K., Pattedkan, H., Divakar, S., Kadimi, U.S. 2010: Chemical composition of *Nigella sativa* L. seed extracts obtained by supercritical carbon dioxide. *Journal of food science and Technology*, 47: 598-605
- Tomas, M., Capanoglu, E., Bahrami, A., Hosseini, H., Akbari-Alavijeh, S., Shaddel, R., Jafari, S.M. 2022: The direct and indirect effects of bioactive compounds against coronavirus. *Food Frontiers*, 3(1): 96-123.
- Villalobos-Castillejos, F., Granillo-Guerrero, V.G., Leyva-Daniel, D.E., Alamilla-Beltran, L., Gutierrez-Lopez, G.F., Monroy-Villagrana, A. 2018: Fabrication of nanoemulsions by microfluidization. In *Nanoemulsions* (pp. 207-232). Academic Press.
- Weinbreck, F., Minor, M., De Kruif, C.G. 2004: Microencapsulation of oils using whey protein/gum Arabic coacervates. *Journal of Microencapsulation*, 21: 667-679.



**Figure 1:** The morphology of micro emulsion moringa oil (A) and micro emulsion black cumin oil (B).



**Figure 2:** The particle structure images of freeze-dried micro emulsion moringa oil (A) and micro emulsion black cumin oil (B)



**Table 1:** Particle size and zeta potential of micro emulsion moringa oil (MeMO) and micro emulsion black cumin oil (MeBCO)

%	Tested Parameter	MeMO	MeBCO
5	Particle size	442.5±21.8	312.5±16.68
	Polydispersity Index (PDI)	0.65±0.02	0.444±0.05
	Zeta potential (mV)	-31.4±0.1	-32.1±0.6
	% separation	--	--
10	Particle size	502.9±30.93	384.6±56.02
	Polydispersity Index (PDI)	0.59±0.035	0.59±0.053
	Zeta potential (mV)	-35.6±2.4	-32.7±0.62
	% separation	--	--
15	Particle size	541.9±39.23	443.7±20.22
	Polydispersity Index (PDI)	0.52±0.036	0.45±0.025
	Zeta potential (mV)	-33.7±0.56	-34.4±0.7
	% separation	--	23
20	Particle size	550.7±22.9	642.1±19.27
	Polydispersity Index (PDI)	0.54±0.017	0.39±0.011
	Zeta potential (mV)	-41.8±0.69	-37.0±0.6
	% separation	35±1.58	65±2.56

Data are reported as mean ± standard deviations of three replicates.

**Table 2:** Encapsulation efficiency (EE) % of freeze-dried micro emulsion moringa oil (MeMO) and micro emulsion black cumin oil (MeBCO).

MeMO (%)				MeBCO (%)			
5	10	15	20	5	10	15	20
80.3±2.58	73.5 <sup>b</sup> ±1.58	60.6 <sup>c</sup> ±3.24	50 <sup>d</sup> ±1.58	82.4 <sup>a</sup> ±1.78	75.6 <sup>b</sup> ±1.58	62.3 <sup>c</sup> ±2.51	50.4 <sup>d</sup> ±1.74

Data are reported as mean ± standard deviations of three replicates. Values with different letters on the same columns represent statistical differences according to a one-way analysis of variance (p<0.05).

**Table 3:** Time (min) needed to reach pH 5.2 and pH 4.6 during fermentation of supplemented milk with different ratio of encapsulated moringa and black cumin oils.

%	YFMO		YFBO	
	pH 5.2	pH 4.6	pH 5.2	pH 4.6
Control	85 <sup>c</sup> ±2.35	145 <sup>c</sup> ±2.35	85 <sup>c</sup> ±2.36	145 <sup>c</sup> ±1.65
2	110 <sup>d</sup> ±2.98	165 <sup>d</sup> ±5.23	115 <sup>d</sup> ±2.47	180 <sup>d</sup> ±1.65
4	120 <sup>c</sup> ±3.25	180 <sup>c</sup> ±3.54	125 <sup>c</sup> ±1.89	200 <sup>c</sup> ±1.98
6	135 <sup>b</sup> ±1.45	195 <sup>b</sup> ±2.36	140 <sup>b</sup> ±1.58	210 <sup>b</sup> ±2.89
8	145 <sup>a</sup> ±2.51	210 <sup>a</sup> ±2.35	150 <sup>a</sup> ±1.36	240 <sup>a</sup> ±2.36

YFMO: yoghurt fortified with encapsulated moringa oil.

YFBO: yoghurt fortified with encapsulated black cumin oil.

Data are reported as mean ± standard deviations of three replicates. Values with different letters on the same columns represent statistical differences according to a one-way analysis of variance (p<0.05).

**Table 4:** The apparent viscosity of fortified yoghurt with encapsulated moringa and black cumin oils

(%)	Shearing time (min)									
	YFMO					YFBO				
	0.0	0.5	1.0	1.5	2.0	0.0	0.5	1.0	1.5	2.0
Control	58.0 <sup>e</sup> ±1.47	46.0 <sup>e</sup> ±1.28	38.0 <sup>e</sup> ±1.22	29.0 <sup>e</sup> ±1.18	20.0 <sup>e</sup> ±1.15	46.0 <sup>e</sup> ±1.28	31.0 <sup>e</sup> ±1.20	28.0 <sup>e</sup> ±1.18	26.0 <sup>e</sup> ±1.16	22.9 <sup>e</sup> ±1.11
2	69.0 <sup>d</sup> ±1.54	64.0 <sup>d</sup> ±1.58	56.0 <sup>d</sup> ±1.48	45.0 <sup>d</sup> ±1.27	34.0 <sup>d</sup> ±1.21	51.0 <sup>d</sup> ±1.44	49.0 <sup>d</sup> ±1.32	37.0 <sup>d</sup> ±1.22	34.0 <sup>d</sup> ±1.21	31.2 <sup>d</sup> ±1.20
4	80.0 <sup>c</sup> ±2.12	77.0 <sup>c</sup> ±1.95	70.0 <sup>c</sup> ±1.88	60.0 <sup>c</sup> ±1.50	48.0 <sup>c</sup> ±1.30	77.0 <sup>c</sup> ±1.98	68.0 <sup>c</sup> ±1.53	56.0 <sup>c</sup> ±1.49	43.0 <sup>c</sup> ±1.25	40.0 <sup>c</sup> ±1.24
6	91.0 <sup>b</sup> ±2.64	87.0 <sup>b</sup> ±2.54	79.0 <sup>b</sup> ±1.98	68.0 <sup>b</sup> ±1.53	56.0 <sup>b</sup> ±1.48	84.0 <sup>b</sup> ±2.55	77.0 <sup>b</sup> ±1.96	64.6 <sup>b</sup> ±1.59	52.0 <sup>b</sup> ±1.44	49.1 <sup>b</sup> ±1.31
8	100 <sup>a</sup> ±2.88	95 <sup>a</sup> ±2.85	87 <sup>a</sup> ±2.54	77 <sup>a</sup> ±1.95	65 <sup>a</sup> ±1.55	93.0 <sup>a</sup> ±2.83	85.6 <sup>a</sup> ±2.48	72.9 <sup>a</sup> ±1.91	60.5 <sup>a</sup> ±1.52	58.5 <sup>a</sup> ±1.48

Data are reported as mean ± standard deviations of three replicates. Values with different letters on the same columns represent statistical differences according to a one-way analysis of variance (p<0.05).

**Table 5:** pH and acidity (TA) of yoghurt fortified with encapsulated moringa and black cumin oil.

Treatments	TA during storage (%)				pH values during storage			
	Fresh	3 days	7 days	14 days	Fresh	3 days	7 days	14 days
Control	0.82 <sup>ab</sup> ±0.014	0.90 <sup>a</sup> ±0.017	1.08 <sup>a</sup> ±0.021	1.26 <sup>a</sup> ±0.027	4.66	4.48	4.23	4.07
YFMO	0.84 <sup>a</sup> ±0.015	0.86 <sup>b</sup> ±0.016	0.95 <sup>b</sup> ±0.018	1.13 <sup>b</sup> ±0.025	4.59	4.55	4.40	4.27
YFBO	0.80 <sup>b</sup> ±0.012	0.83 <sup>c</sup> ±0.015	0.91 <sup>c</sup> ±0.017	0.99 <sup>c</sup> ±0.019	4.52	4.50	4.42	4.28

Data are reported as mean ± standard deviations of three replicates. Values with different letters on the same columns represent statistical differences according to a one-way analysis of variance (p<0.05).

**Table 6:** Effect of fortified yoghurt with encapsulated moringa and black cumin oils on the coliform and moulds & yeasts counts.

Organisms	Control	YFMO (%)				YFBO (%)			
		2	4	6	8	2	4	6	8
Coliform	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mould & yeast	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: Not detected

**Table 7:** Organoleptic properties of fortified yoghurt with encapsulated moringa and black cumin oils.

Properties	Control	YFMO (%)				YFBO (%)			
		2	4	6	8	2	4	6	8
Flavor (60)	56 <sup>a</sup> ±2.3	51 <sup>cd</sup> ±2.0	54 <sup>b</sup> ±2.2	50 <sup>d</sup> ±2.0	48 <sup>e</sup> ±1.9	53 <sup>bc</sup> ±2.2	56 <sup>a</sup> ±2.3	52 <sup>c</sup> ±2.2	50 <sup>d</sup> ±2.0
Body & Texture (30)	27 <sup>ab</sup> ±1.3	26 <sup>b</sup> ±1.3	28 <sup>a</sup> ±1.3	24 <sup>c</sup> ±1.3	20 <sup>e</sup> ±1.2	26 <sup>b</sup> ±1.3	28 <sup>a</sup> ±1.3	22 <sup>d</sup> ±1.2	20 <sup>e</sup> ±1.2
Color & Appearance (10)	8 <sup>a</sup> ±0.2	7 <sup>b</sup> ±0.1	8 <sup>a</sup> ±0.2	7 <sup>b</sup> ±0.12	5 <sup>e</sup> ±0.1	8 <sup>a</sup> ±0.2	8 <sup>a</sup> ±0.2	6 <sup>c</sup> ±0.1	5 <sup>e</sup> ±0.1
Total score (100)	91 <sup>a</sup> ±3.1	84 <sup>c</sup> ±3.0	90 <sup>a</sup> ±3.0	81 <sup>d</sup> ±2.9	71 <sup>f</sup> ±2.8	87 <sup>b</sup> ±3.0	92 <sup>a</sup> ±3.1	80 <sup>d</sup> ±2.9	75 <sup>e</sup> ±2.9

Data are reported as mean ± standard deviations of three replicates. Values with different letters on the same columns represent statistical differences according to a one-way analysis of variance (p<0.05).

## الخصائص الفيزيائية والكيميائية لزبادي وظيفي مدعم بزيت المورينجا وحب البركة المكبسلة

أحمد فتحي عبدالوهاب الشيخ<sup>1</sup>، ممدوح أحمد عمر<sup>1</sup>، علي ابراهيم منصور<sup>2</sup>، محمود عبد اللاه أحمد<sup>2</sup>، دينا مصطفى محمد<sup>3</sup>، تامر محمد المسيري<sup>4</sup>

<sup>1</sup> قسم الألبان، كلية الزراعة بالقاهرة، جامعة الأزهر، القاهرة، مصر

<sup>2</sup> قسم الألبان، كلية الزراعة، جامعة الأزهر، أسيوط، مصر

<sup>3</sup> قسم التغذية وعلوم الأطعمة، معهد بحوث الصناعات الغذائية والتغذية، المركز القومي للبحوث، الجيزة، مصر

<sup>4</sup> قسم الألبان، المركز القومي للبحوث، الجيزة، مصر

\* البريد الإلكتروني للباحث الرئيسي:

### الملخص العربي:

تعتبر الزيوت الطبيعية من الأهمية بمكان في التأثير الأيجابي على الناحية الصحية للإنسان ولكن إستخدامها في صورتها الطبيعية قد تحدث بعض المشاكل التصنيعية والحسية والكيميائية مما يقلل من فوائدها الصحية. الهدف من هذا البحث هو انتاج زبادي حيوي مدعم بزيت المورينجا وزيت حبة البركة المكبسلة. حيث تم تصنيع الكبسولات الدقيقة من زيت المورينجا وزيت حبة البركة باستخدام maltodextrin (MD) المالتوديكسترين، ومركز بروتين اللبن والصمغ العربي (GA) كوامل حاملة باستخدام التجفيف بالتجميد. تمت دراسة خصائص المستحلبات الدقيقة حيث كشف توصيف المستحلبات الدقيقة بتركيزات مختلفة من زيت المورينجا وزيت حبة البركة عن مستحلبات مستقرة ذات شحنات كهربائية (-31.4 إلى -41.8 mV لزيت المورينجا و -32.1 إلى -37.0 mV لزيت حبة البركة). تراوحت أحجام الجسيمات من 442.5 إلى 550.7 نانومتر لزيت المورينجا و 312.5 إلى 642.1 نانومتر لزيت حبة البركة. كانت كفاءة التغليف أعلى عند تركيز الزيت 5٪ ولكنها انخفضت بتركيزات أعلى. إن إضافة زيت المورينجا وزيت حبة البركة المكبسلة إلى اللبن يؤثر على تخميره ولزوجته ودرجة الحموضة وارقم ال pH. أظهر الزبادي الحيوي المدعم بالزيوت غياب البكتيريا الضارة. أشار التقييم الحسي إلى أن التركيز الأمثل بنسبة 4٪ لكلا النوعين من الزبادي المدعم قامت بتحسين النكهة والملمس. بشكل عام، أدت إضافة الزيوت المكبسلة إلى تحسين القيمة الغذائية والفوائد الصحية المحتملة للزبادي.

**الكلمات الاسترشادية:** الزبادي، الكبسولة الدقيقة، التجفيف بالتجميد، المورينجا اوليفيرا، حبة البركة.