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Development of flavored yoghurt fortified with microcapsules of triple omega 3- 6- 9 for preventing neurotoxicity induced by aluminum chloride in rats

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Abstract

Microcapsules of omega-3, -6, and -9 (fish, wheat germ, and olive oils at ratio 2:1:1) were microencapsulated using maltodextrin (MD), whey protein concentrate, gum Arabic (GA), and lactoferrin (LF) as carrier agents. The microcapsules were added to yoghurt that previously flavored by addition of orange essential oil. The characteristics of either the microcapsules or the flavored yoghurt were studied. The protective effect of the microcapsules and the flavored yoghurt in which these microcapsules were added against neurotoxicity mediated by aluminum chloride was assessed. The microcapsules prepared from MD: GA: LF and omega-3, -6, and -9 at ratio 4:1 showed the highest encapsulation efficiency with less separation%, and zeta potential. The flavored yoghurt containing microcapsules recorded high scores in the sensorial evaluation, showed protective effect against aluminum chloride-mediated neurotoxicity, enhanced brain dopamine, and 5-hydroxytriptamine, suppressed the elevation of brain acetylcholinesterase activity and attenuated the oxidative stress markers.

Novelty impact statement

The flavored yoghurt containing triple omega-3, -6, and -9 microcapsules recorded high sensory attributes, showed neuroprotective effect against the aluminum chloride, attenuated the oxidative stress markers, and enhanced brain dopamine and serotonin.

1 | INTRODUCTION

Unsaturated fatty acids (omega-3, -6, and -9) have a vital role in promoting human health. It is well known that they are not naturally synthesized in human body, except omega-9 (Castejón et al., 2021). Fish oil is rich in omega-3 (α-Linolenic acid), wheat germ oil is rich in omega-6 (Linoleic acid), and omega-9 (Oleic acid) is mainly present in olive oil (Balić et al., 2020; Donovan et al., 2020). Some kinds of dietary patterns result in elevated consumption of omega-6 fatty acid than omega 3 fatty acid resulting in obesity and high risk of cardiovascular disease. Therefore, the World Health Organization (WHO) and the American Heart Association (AHA) recommended the consumption of omega-3 fatty acid more than omega-6 (Simopoulos, 2016).

Triple omega-3, -6, and -9, with a higher level of omega 3 than omega 6, is considered of great importance not only as a nutraceutical but also for the development of functional foods. But unfortunately, hydrophobicity and high sensitivity to oxidation, promote the formation of oxidation products implying undesirable odors and flavors, consequently make the development of functional foods with triple omega a great challenge (Vieira et al., 2020). The microencapsulation technology represents an efficient technique not only for protecting the triple omega from oxidation and preserving their stability for a longer period, but also to achieve the possibility of fortifying the food products specially the dairy products and beverages with these biological active compounds (Castejón et al., 2021).

Aluminum (Al) is a neurotoxic substance that affects humans through contaminated food and water in addition to particle inhalation (Igbokwe et al., 2019). Aluminum was reported to be among the factors that mediated the neurodegenerative diseases such as Alzheimer's disease (Rather et al., 2018), mainly through the accumulation in the brain and generation of reactive oxygen species (ROS; Al-Amin et al., 2019). The validation of neuroprotective agents is of major interest, especially that until now there are no curatives for neurodegenerative diseases (Bilgic et al., 2018).

This study was conducted to achieve multiple purposes. First was the production of triple omega-3, -6, and -9 microcapsules to be added to dairy product like yoghurt. Second was the addition of orange peels oil to yoghurt in order to mask the undesirable fishy taste of fish oil. Third was to characterize the triple omega microcapsules and the flavored yoghurt containing microcapsules. Fourth was to study the possible protective effect of these microcapsules and the flavored yoghurt containing microcapsules against neurotoxicity and oxidative stress mediated by aluminum chloride in intoxicated rats.

2 | MATERIALS AND METHODS

2.1 | Materials

Orange peels (*Citrus sinensis*) were obtained from a local market in Egypt. Fish Oil (Norwegian fish oil) was purchased from the Amazon website. Wheat germ and olive oils were provided from the oil extraction unit in National Research Centre (NRC) Egypt. Whey protein concentrate (WPC, 80% protein), maltodextrin (MD), and gum Arabic (GA) were purchased from Alfasol Co., Turkey. Low-heat skimmed milk powder (USA) (34% protein, 51%, fat 8.2%, lactose, 1.2% ash and 4% moisture) was purchased from a local market in Egypt. Starter strains of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were obtained from dairy microbiology Lab., NRC, Egypt. Lactoferrin (LF), from bovine milk ≥85%, was purchased from Sigma-Aldrich Co. (St. Louis, USA). The used chemicals for analysis were of analytical grade and were purchased from Sigma-Aldrich Co. (St. Louis, USA).

2.1.1 | Animals

Male Wistar rats (geriatric rats, 18 months old) of 354.37 ± 37.75 g as mean ± *SD* were used in this study. Animals were obtained from the animal house of the NRC, Cairo, Egypt. Animals were kept individually in metabolic stainless-steel cages; water and food were given ad-libitum. This study has been carried out as a part of an internal project in the NRC, Cairo, Egypt. This project was approved by the Medical Research Ethics Committee, NRC, Cairo, Egypt with approval number 19182. The study followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

2.1.2 | Animals' diet

Balanced diet (12% protein supplemented from casein, 10% corn oil, 10% sucrose, 58.5% maize starch, 5% fiber, 3.5% salt mixture, and 1% vitamin mixture), salt, and vitamin mixtures were prepared in accordance with AIN-93 (Reeves et al., 1993).

2.2 | Methods

2.2.1 | Fatty acids composition

Fatty acids composition of oils was determined according to Zahran and Tawfeuk (2019).

2.2.2 | Orange essential oil extraction

Orange essential oil was extracted from the peels by hydrodistillation technique (Golmohammadi et al., 2018; Li et al., 2014).

2.2.3 | Gas chromatographic-mass spectrometric analysis (GC/MS) of orange oil

About 2 µl of orange oil was used. The analysis was carried out by using a coupled gas chromatography, Hewlett-Packard model (5,890)/mass spectrometer Hewlett-Packard-MS (5,970). The ionization voltage was 70 eV, mass range *m/z* 39–400 a.m.u. The oven temperature was maintained initially at 40°C for 5 min., and then programmed from 40 to 250°C at a rate of 4°C/min. Helium was used as the carrier gas, at flow rate of 1.1 ml/min. The injector and detector temperatures were 220 and 250°C, respectively.

2.2.4 | Preparation of triple omega-3, -6, and -9 microcapsules

Six different formulations of emulsions, mentioned in Table 1, containing triple omega-3, -6, and -9 (2:1:1), were prepared using maltodextrin (MD), GA, and WPC as carrier agents. All emulsions were dried using a spray dryer as described by Abdel-Razek et al. (2018; Figure 1).

2.2.5 | Characterizations of the emulsions

Emulsions stability

Twenty-five microliters of emulsion was kept in a covered cylinder at ambient temperature for 24 hr. The separation layer was measured. The percent of separation was calculated by Equation (1):

$$\text{Percent of separation} = (H1/H0) \times 100 \quad (1)$$

TABLE 1 Emulsions formulations' composition

Formulation code	Carrier agents				Carrier agents: core*
	MD (g)	GA (g)	WPC (g)	LF (mg/ml)	
F1	75	0	25	30	2:1
F2	75	0	25	30	4:1
F3	75	12.5	12.5	30	2:1
F4	75	12.5	12.5	30	4:1
F5	75	25	0	30	2:1
F6	75	25	0	30	4:1

*Core: mixture of triple omega sources, fish oil (omega 3), wheat germ oil (omega 6), and olive oil (omega 9) at ratio 2:1:1.

Abbreviations: GA, gum Arabic; LF, lactoferrin; MD, maltodextrin; WPC, whey protein concentrate.

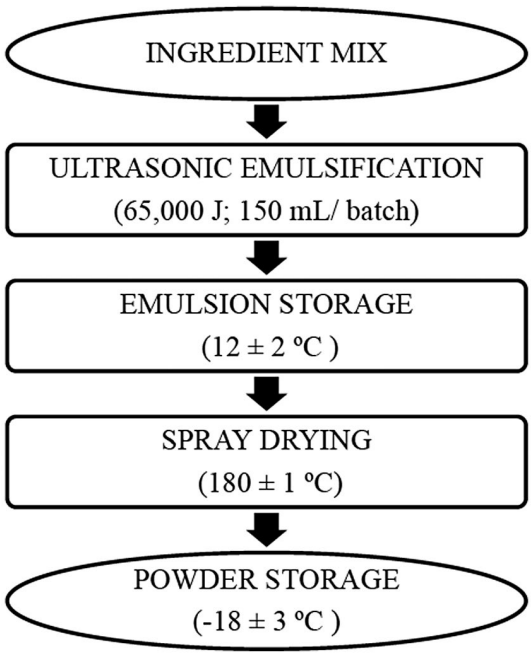

FIGURE 1 Flow diagram of microcapsules production using spray dryer

where: H0 is the initial volume of the emulsion and H1 is the volume of the separation layer.

Particle size and zeta potential of the emulsions

Particle size and zeta potential of emulsions were measured by dynamic light scattering (Zeta sizer var, 704 instruments, Malvern Instruments, Malvern, UK) as previously described by El-Said et al. (2018).

2.2.6 | Characterizations of the microcapsules

Encapsulation efficiency (EE)

Encapsulation efficiency was evaluated according to Bae and Lee (2008). In test tube, 15 ml of hexane was mixed with 1.5 g of microcapsules powder. The mixture was vortexed for 2 min to remove the unencapsulted oil. Whatman filter paper No. 1 was used to filter the previous mixture. Twenty ml of hexane was used to rinse the collected powder for three times. Hexane was evaporated at 60°C to obtain a fixed weight. By Equation (2), the encapsulation efficiency was calculated:

$$EE = (TO - SO/TO) \times 100 \quad (2)$$

where TO is the total oil and SO is the unencapsulated oil.

Surface morphology

Scanning electron detector microscope was used to observe the microcapsules with energy dispersive X-ray, *SEM*, and EDX Leo 440i 6,070 (LEO Electron Microscopy, Oxford, England) operated at 15 kV and electron beam current of 100 pA.

2.2.7 | Preparation of flavored yoghurt fortified with triple omega-3, -6, and -9

The best type of microcapsules was chosen for yoghurt fortification. Yoghurt was prepared from reconstituted skim milk powder (14% total solids) and different levels of triple omega-3, -6, and -9 oils (1, 2, 3, and 4%). All the batches were heated to 90°C for 10 min then cooled to 45°C, and then 0.1% orange oil was added. The starter culture was inoculated at the rate of 3 g/100 ml milk to each batch and filled into 100 ml plastic cups. Incubation was carried out at 45 ± 1°C for about 3 hr. Once the desired consistency was obtained, set yoghurt cups were transferred to refrigerated storage of 4–7°C for 14 days.

Physicochemical properties of the flavored yoghurt fortified with triple omega-3, -6, and -9

Digital pH-meter (JENWAY 3,505) was used to measure the pH. Titratable acidity was determined according to AOAC (2007). The apparent viscosity of yoghurt samples was observed by a dynamic viscometer (Brookfield Model-LV; Brookfield Engineering Laboratory, Stoughton, USA) at a speed from 0.3 to 100 rpm. The

% syneresis was determined by Achanta et al. (2007). The syneresis percent was calculated by Equation (3):

$$\text{Percent of syneresis} = VE/Y \quad (3)$$

Here in, VE is the supernatant weight and Y is the yoghurt weight.

Sensory evaluation of the flavored yoghurt fortified with triple omega-3, -6, and -9

The sensorial valorization was done by 20 panelists and the score was based on the 5-point scoring form (1; Very Bad, 2; Bad, 3; Normal, 4; Good, 5; Excellent) for color, taste and aroma, texture, and overall acceptability (Lawless & Heymann, 2010).

2.2.8 | Induction of $AlCl_3$ neurotoxicity

$AlCl_3$ solution was made freshly for oral administration. $AlCl_3$ was dissolved in distilled water and administered orally in a dose of 100 mg/kg b.wt rat (1 ml to rat daily for 5 weeks) according to Ahmed et al. (2020); Saleem et al. (2021).

2.2.9 | Design of the animals' study

Thirty rats were divided, after 1-week acclimatization, into five groups ($n = 6$) as follows:

G1: The control group, where rats were daily orally given distillated water.

G2: Aluminum chloride group (Al), where rats were daily orally given anhydrous aluminum chloride.

G3: microcapsules (M) + aluminum chloride group, where rats were daily orally given the microcapsules (120 mg containing 30 mg oils) beside the aluminum chloride.

G4: Control yoghurt (CY) + aluminum chloride group, where rats were daily orally given 1ml of control yoghurt beside the aluminum chloride.

G5: Fortified yoghurt (FY, sample T3) + aluminum chloride group where rats were daily orally given 1 ml of T3 (containing 30 mg oils) beside the aluminum chloride.

All rats were fed on a balanced diet all over the study period. During the experiment, food intake was recorded daily. At the end of the study, total food intake, body weight gain, and feed efficiency ratio were calculated.

Biochemical analysis

After the experimental period (5 weeks), rats were sacrificed under anesthesia and brain was immediately separated from each rat and weighed. The homogenate of each rat's brain (hippocampus and cortex) was immediately analyzed for MDA, catalase activity, Nitric oxide (NO), reduced glutathione, acetylcholinesterase, and butrylcholinesterase according to Ohkawa et al. (1979), Aebi (1984), Montgomery and Dymock (1961), Sedlak and Lindsay (1968), Ellman et al. (1961), and Vaisi-Raygani et al. (2007), respectively. Also, brain superoxide dismutase (SOD), dopamine (DA), and 5-hydroxytraptamine (5-HT; serotonin) were determined using Eliza kits (Sunlong Biotech, China) in accordance with the manufacturer's instructions.

2.2.10 | Statistical analysis

Statistical analysis was accomplished via the one-way analysis of variance ANOVA followed by Duncan's test using SPSS version 16. The results were expressed as mean ± standard error (SE). The statistical significance of the difference was taken as $p \leq .05$.

3 | RESULTS AND DISCUSSION

Analysis of fatty acids (Table 2) of fish, wheat germ, and olive oils showed 15 fatty acids. The main fatty acids in fish oil were oleic acid, DHA, and EPA. The most prevailing fatty acid in wheat germ oil was linoleic. Olive oil analysis showed that oleic acid concentration is the highest percentage among fatty acids. DHA and EPA were absent in wheat germ oil and olive oil. The omega 3 contents were 21.59, 3.24, and 0.76% and the omega 6 contents were 7.32%, 56.13%, and 10.19%, in fish, wheat germ, and olive oils, respectively. Olive oil had the highest content of omega 9.

Concerning the orange essential oil, results showed that the total oil yield was 1.1/100 g dm. Limonene was the most abundant aromatic compounds in orange oil. Limonene is one of the most common compounds found in orange essential oil and responsible for its

TABLE 2 Fatty acids methyl esters composition (FAME) % of fish oil, wheat germ oil, olive oil

FAME %	Fish oil	Wheat germ oil	Olive oil
C 14:0 *Myristic acid*	4.11	0.11	0.09
C16:0 *Palmitic acid*	10.26	7.94	10.99
C16:1 n7 Palmitoleic acid	5.17	6.36	0.18
C16:1 n9 *Hypogeic acid*	2.13	27.2	0.95
C17:0 *Margaric acid*	0	0	0.13
C18:0 Stearic acid	6.99	9.3	14.36
C18:1 n9c *Oleic acid*	20.94	11.73	51.56
C18:1 n7 t *Vaccenic acid*	6.19	3.59	2.02
C18:2 n6c *Linoleic acid*	3.85	55.22	9.79
C18:2 n6 t *Linolelaidic acid*	3.47	0.91	0.4
C18:3 n3 α-*Linolenic acid*	1.08	3.24	0.76
C20:0 *Arachidic acid*	2.64	0.12	0.43
C20:1 n9 *Gondoic acid*	8.04	5.21	0.26
C20:5 n3 (EPA) *Eicosapentaenoic acid*	10.15	0	0
C22:6 n3 (DHA) *Docosahexaenoic acid*	10.36	0	0

flavor. This distinctive flavor makes it a favorite flavor for many food products (Gültepe, 2020).

Concerning the emulsion stability (Table 3), results revealed that the prepared emulsions using MD and GA were more stable than those prepared using MD and WPC. The later showed higher separation %, foam, and small separating layer after 24 hr. This result was unexpected as whey protein is a strong emulsifier. This may be due to unfolding of the protein molecules on the surface of the droplets which led to increased protein–protein interaction and aggregated during emulsification and thus the emulsification property may be lost and the emulsion stability is decreased (McClements & Jafari, 2018).

Concerning zeta potential and particle size, results revealed as shown in Table 3 that the formulations F5 and F6 had the least zeta potential (−47.83 and −57.451 mV) and exhibited stability more than other formulations. Villalobos-Castillejos et al. (2018) mentioned that emulsions exhibit coagulate when zeta potential within the range 30 to −30 mV, while emulsions are more stable when zeta potential is higher than +30 mV or lower than −30 mV. This interprets the stability of the prepared emulsions using GA. Also, the prepared emulsions using MD and GA were smaller in size and with lower zeta potential compared to that prepared using MD, GA, and WPC.

TABLE 3 Characterizations of emulsions

Emulsions formulations	% Separation	Particle size (nm)	Zeta potential (mv)
F1	1.71 ± 0.08	235.23 ± 0.76	−29.22 ± 1.30
F2	2.05 ± 0.07	289.24 ± 1.24	−30.96 ± 1.19
F3	0.68 ± 0.04	271.78 ± 1.67	−34.29 ± 1.16
F4	–	257.66 ± 1.07	−39.34 ± 1.13
F5	–	410.85 ± 1.20	−46.83 ± 1.53
F6	–	748.74 ± 0.88	−57.45 ± 1.77

Note: F1 = carrier agents (MD and WPC 3:1): core was 2:1, F2 = carrier agents (MD and WPC 3:1): core was 4:1, F3 = carrier agents (MD:GA:WPC 3:0.5:0.5): core was 2:1, F4 = carrier agents (MD:GA 3:0.5:0.5): core was 4:1, F5 = carrier agents (MD and GA 3:1) : core was 2:1, F6 = carrier agents (MD:GA 3:1): core was 4:1

Concerning the characterization of the microcapsules, results (Figure 2) showed that the microcapsulation was performed successfully using spray drying technique and the encapsulation efficiency (EE) of microcapsules varied from 52.63% to 88.61%. EE was comparatively higher in F5 and 6 than other formulations. It was noticed that the EE of microcapsules significantly increased with the elevation of GA. This may be attributed to the high viscosity of emulsion, which in turn encourages an increased in the drying speed. EE of microcapsules significantly decreased with the increase in WPC (F1 and F2). In this respect, Charve and Reineccius (2009) prepared microcapsules using modified starch, GA, and whey protein and observed that modified starch showed the highest oil retention.

The surface microstructures (SM) of different formulations' microcapsules (F1 to F6) are shown in Figure 3. The particles had a spherical shape and did not show any cracks. Which indicates that there was no permeability to the entry of gases, and thus indicates the ability of these microcapsules to retain of bioactive compounds. Also, the microparticles morphology did not affect by different wall materials. Regarding to the SM, F5, and F6 microcapsules, which were prepared using GA and MD, were the best formulations.

In this study, flavored yoghurt was chosen to be fortified with the triple omega microcapsules because it is a nutritious, grainy and easy-to-eat food. The Physicochemical properties of the flavored yoghurt containing microcapsules showed low titratable acidity (TA) which is considered a significant factor for assessment of yoghurt quality and to what extent accepted by consumer. TA values of the flavored yoghurt containing microcapsules decreased significantly ($p < .05$) with increasing microcapsules content, and these values increased during cold storage for all samples (Table 4). The increase in TA may be due to the increase in microorganism's activity

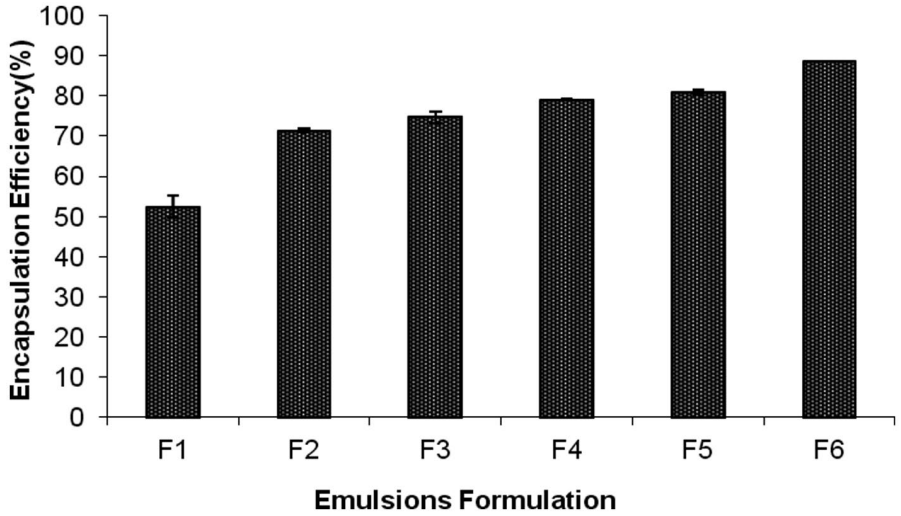

FIGURE 2 %Encapsulation efficiency of the triple omega (3, 6, and 9) microcapsules

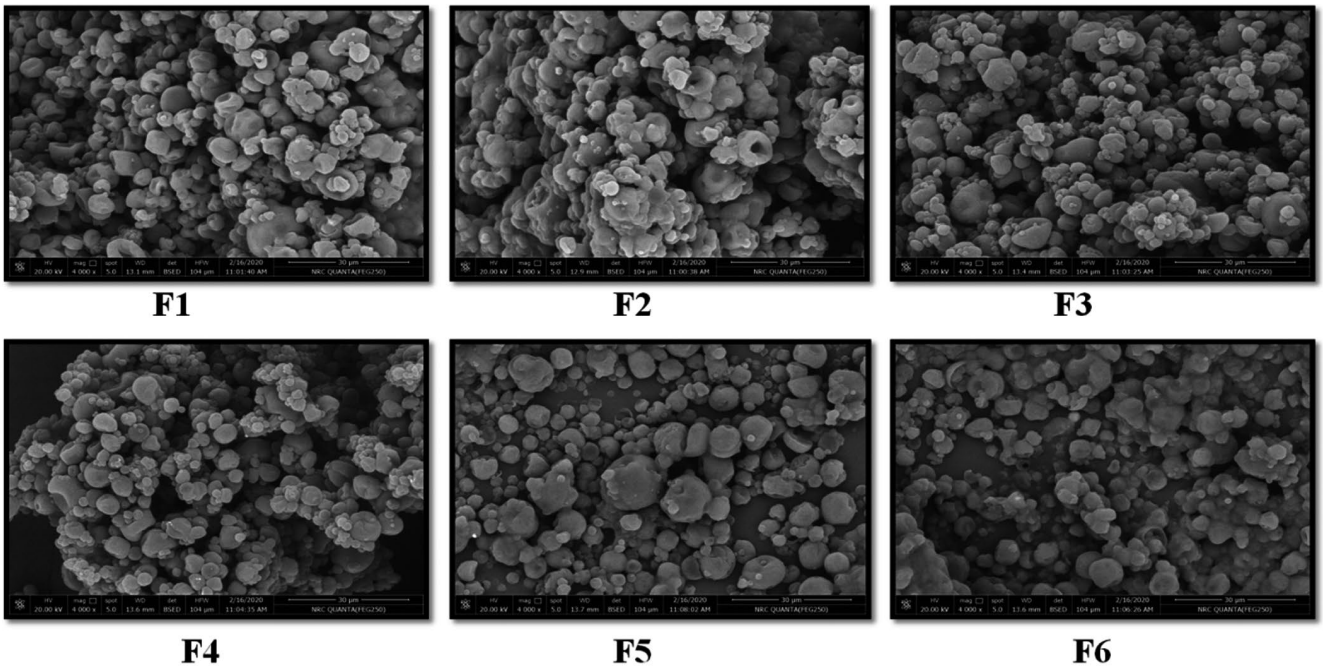

FIGURE 3 Surface microstructures of the triple omega (3, 6, and 9) microcapsules

(Kailasapathy, 2002). Moreover, the pH values of the flavored yoghurt containing microcapsules increased significantly ($p < .05$) with increasing microcapsules, while all values decreased during the storage. These results coincide with Perina et al. (2015), who reported that vegetable oil emulsion had a significant effect on pH value of yoghurt.

The syneresis values reduced significantly with increasing microcapsules content in yoghurt and during the storage. This may be due to the increase in yoghurt total solids as a result of the presence of MD and GA which decrease the porosity of yoghurt, accelerate the absorption of water, improve the stability of yoghurt, and minimize the syneresis rate (Ghorbanzade et al., 2017). Reduction of pH values of yoghurt samples during the storage is the one important factor that affects the syneresis. Earlier studies showed that reduction of pH is associated with the decreased bonding energy between whey proteins and casein network, which leads to a decrease in the syneresis rate (Akgun et al., 2017; Zhong et al., 2018).

Concerning the viscosity, Figure 4 showed that the viscosity values of the refrigerated fortified yoghurt were significantly higher than that of the plain yoghurt (control), (T4> T3> T2> T1> plain yoghurt, respectively). This may be due to the presence of MD and GA which increased the total solids content of yoghurt.

The sensory evaluation is important because it shows to what extent the flavored yoghurt containing microcapsules is accepted for consumers in this respect the taste, texture, and overall acceptability were recorded (Table 5). In terms of texture, control yoghurt had the lowest score, followed by T1, T2, T3, and, T4 respectively. This is related to the high viscosity which affects the texture. The flavored yoghurt had the highest score in taste and aroma compared to control yoghurt because the addition of orange peels oil to yoghurt masked any undesirable taste. Zhong et al. (2018) recommended using flavored yoghurt to improve the total acceptability of yoghurt containing fish oil/or yzanol nanoemulsion. It can be deduced that the flavored yoghurt containing microcapsules was accepted by consumers specially after adding orange peels oil which is capable to mask any undesirable taste and odor.

Experimental exposure to Al toxicity induces biochemical alterations and oxidative stress in the brain of rat model that mimics human Alzheimer's disease. Thus, any neuroprotective agent that can suppress the oxidative stress and the biochemical alterations may protect the brain from the development of neurodegenerative diseases (Aboelwafa et al., 2020).

Results of this study revealed no significant difference ($p > .05$) between groups in the total food intake (Table 6). However, it was notable that the administration of aluminum chloride (Al) was accompanied with decreasing in the body weight and this is agreed with Taïr et al. (2016). Also, the administration of triple omega microcapsules along with aluminum chloride resulted in decreasing in the body weight due to the presence of fish oil that can cause loss of weight as confirmed by Al-Attar and Al-Rethea (2017). However, it is evident that fortified yoghurt with triple omega microcapsules combated the decreasing in the body weight subsequent to aluminum chloride exposure. The control group was significantly high in feed efficiency ratio than other groups, while Al group recorded the less feed efficiency ratio.

On one hand, the brain' (hippocampus and cortex homogenate) neurotransmitters (5-HT and DA) significantly reduced in the $AlCl_3$ treated rats by 50.19 and 32.43%, respectively (Figure 5). On the other hand, the administration of the triple omega microcapsules, the plain yoghurt, or the fortified yoghurt significantly elevated

TABLE 4 Physiochemical properties of the flavored yoghurt fortified with triple omega

Treatments	PH			Titratable acidity %			% Syneresis		
	Storage period (day)								
	Fresh	7	14	Fresh	7	14	Fresh	7	14
Control	$4.63^{e} \pm 0.00$	$4.62^{e} \pm 0.0$	$4.46^{e} \pm 0.01$	$0.96^{a} \pm 0.02$	$1.14^{a} \pm 0.02$	$1.18^{a} \pm 0.01$	$39.42^{a} \pm 0.15$	$37.45^{a} \pm 0.32$	$35.5^{a} \pm 0.53$
T_1	$4.64^{d} \pm 0.01$	$4.63^{d} \pm 0.00$	$4.49^{d} \pm 0.01$	$0.88^{b} \pm 0.05$	$1.09^{a} \pm 0.05$	$1.13^{a} \pm 0.00$	$38.74^{b} \pm 0.38$	$36.31^{b} \pm 0.16$	$33.62^{b} \pm 0.06$
T_2	$4.65^{c} \pm 0.00$	$4.64^{c} \pm 0.00$	$4.53^{c} \pm 0.01$	$0.75^{c} \pm 0.00$	$0.86^{b} \pm 0.02$	$1.05^{a} \pm 0.00$	$36.43^{c} \pm 0.06$	$35.41^{c} \pm 0.41$	$32.63^{c} \pm 0.08$
T_3	$4.68^{b} \pm 0.00$	$4.65^{b} \pm 0.01$	$4.58^{b} \pm 0.04$	$0.58^{d} \pm 0.05$	$0.94^{b} \pm 0.05$	$0.99^{a} \pm 0.199$	$35.4^{d} \pm 0.34$	$33.57^{d} \pm 0.12$	$31.47^{e} \pm 0.32$
T_4	$4.69^{a} \pm 0.02$	$4.66^{a} \pm 0.01$	$4.61^{a} \pm 0.01$	$0.55^{d} \pm 0.01$	$0.89^{b} \pm 0.02$	$0.98^{a} \pm 0.20$	$33.19^{e} \pm 0.01$	$31.21^{e} \pm 0.08$	$29.29^{d} \pm 0.23$

In the same column, different superscript means significant difference ($p < .05$). Control: yoghurt without microcapsules. T1: yoghurt fortified with 1% triple omega (3,6,9). T2: yoghurt fortified with 2% triple omega (3,6,9). T3: yoghurt fortified with 3% triple omega (3,6,9). T4: yoghurt fortified with 4% triple omega (3,6,9).

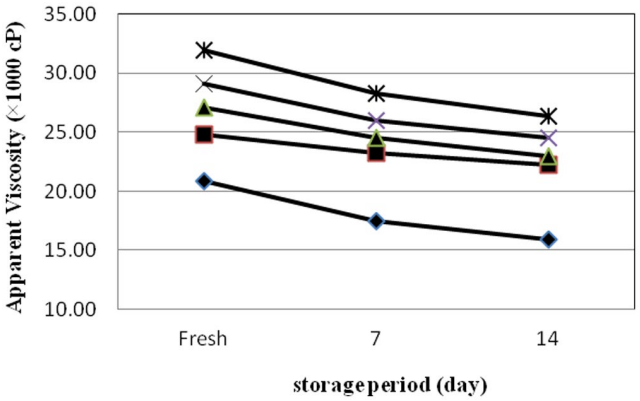

FIGURE 4 The viscosity of yoghurt samples during cold storage (fresh, 7 and 14 days). Control (*): yoghurt without microcapsules. T1 (▲): yoghurt fortified with 1% triple omega (3, 6, and 9) microcapsules. T2 (●): yoghurt fortified with 2% triple omega (3, 6, and 9) microcapsules. T3 (◆): yoghurt fortified with 3% triple omega (3, 6, and 9) microcapsules. T4 (■): yoghurt fortified with 4% triple omega (3, 6, and 9) microcapsules

these neurotransmitters. However, the fortified yoghurt was the most promising in this concern. The preventive effect of yoghurt against the reduction of neurotransmitters may be attributed to the individual molecules produced during the fermentation process such as oleamide and dehydroergosterol which have been known as the agents involved in the reducing of microglial inflammatory responses and neurotoxicity (Ano & Nakayama, 2018). Ano et al. (2018) found that Trp-Tyr (WY) containing peptides from whey protein enhances memory functions in the mice and declared that WY-containing peptides in the fermented dairy products increase dopamine levels by inhibiting the activity of monoamine oxidase-B which are useful in preventing age-related cognitive.

The effect of triple omega beside the mentioned effect of yoghurt may explain the superiority of fortified yogurt in increasing the values of the neurotransmitters (5-HT and DA). It was found that omega-3 is the main agent responsible for increasing dopamine in the brain. So, the intake of omega-3 was recommended by Healy-Stoffel and Levant (2018) who declared that low levels of omega-3 in brain negatively affects the brain dopamine systems and may be a risk factor for some neurodegenerative and neuropsychiatric disorders (Parkinson's disease, schizophrenia, and depression). It was found that the ratio between omega-3 and 6, as one of the food quality criteria, positively affects the mood and cognitive functions (Young et al., 2020). On this basis, the ratio between omega 3 and 6 in the triple omega microcapsules might contribute to the increase in the values of neurotransmitters. Kokras et al. (2020) reported that olive oil has a promising effect against cognitive decline and mental disorder due to its content of biological active substances that increase neurotransmitters.

Elevating of AChE and BChE, the main enzymes that cause the breakdown of cholinesterase in brain, contributes to Alzheimer's disease and the inhibitors of these enzymes are considered as medications of Alzheimer's disease (Sharma, 2019). Results of

TABLE 5 Sensory attributes of the flavored yoghurt fortified with triple omega at first day of storage

Yoghurt samples	Sensory quality score			
	Color	Taste and aroma	Texture	Overall acceptance
Control	$4.94^{a} \pm 0.09$	$3.66^{d} \pm 0.23$	$3.16^{c} \pm 0.21$	$4.39^{b} \pm 0.13$
T1	$4.90^{a} \pm 0.07$	$4.96^{a} \pm 0.09$	$3.28^{c} \pm 0.31$	$4.77^{a} \pm 0.05$
T2	$4.74^{a} \pm 0.15$	$4.16^{c} \pm 0.21$	$4.14^{b} \pm 0.22$	$4.35^{b} \pm 0.13$
T3	$4.78^{a} \pm 0.23$	$4.36^{bc} \pm 0.21$	$4.46^{ab} \pm 0.15$	$4.14^{c} \pm 0.09$
T4	$4.94^{a} \pm 0.09$	$3.98^{bc} \pm 0.15$	$4.58^{a} \pm 0.43$	$4.03^{c} \pm 0.06$

In the same column, different superscript means significant difference ($p < .05$). T1: yoghurt fortified with 1% triple omega (3,6,9). T2: yoghurt fortified with 2% triple omega (3,6,9). T3: yoghurt fortified with 3% triple omega (3,6,9). T4: yoghurt fortified with 4% triple omega (3,6,9).

TABLE 6 Nutritional parameters of the different experimental groups

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Total food intake (g)	Feed efficiency ratio
Control normal	$354.00^{a} \pm 14.51$	$380.67^{b} \pm 12.86$	$26.67^{c} \pm 2.58$	$622.00^{a} \pm 17.07$	$0.04^{c} \pm 0.01$
Al	$354.67^{a} \pm 18.35$	$323.33^{a} \pm 12.73$	$-31.33^{a} \pm 9.11$	$607.67^{a} \pm 25.65$	$-0.05^{a} \pm 0.01$
M + Al	$354.50^{a} \pm 8.89$	$348.00^{ab} \pm 14.09$	$-6.50^{ab} \pm 17.23$	$569.17^{a} \pm 28.06$	$-0.02^{ab} \pm 0.03$
CY + Al	$354.33^{a} \pm 18.16$	$366.83^{ab} \pm 17.28$	$12.50^{bc} \pm 3.14$	$639.00^{a} \pm 27.16$	$0.02^{bc} \pm 0.01$
FY + Al	$354.33^{a} \pm 20.52$	$361.50^{ab} \pm 25.84$	$7.17^{bc} \pm 6.87$	$627.83^{a} \pm 38.78$	$0.01^{bc} \pm 0.01$

Note: In each column same letter means non-significant difference, while different letter means significant difference at 0.05 probability. The data are expressed as mean values ± standard error. Al, rats treated with aluminum chloride; M + Al, rats treated with microcapsules along with aluminum chloride; CY + AL, rats treated with control yoghurt along with aluminum chloride; FY + Al, rats treated with fortified yoghurt along with aluminum chloride.

TABLE 7 Brain parameters of the different experimental groups

Parameters	Control normal	Al	M + Al	CY + Al	FY + Al
Acetylcholine esterase (ng/g tissue)	$0.98^{a} \pm 0.06$	$2.23^{c} \pm 0.07$	$1.72^{b} \pm 0.08$	$2.03^{c} \pm 0.08$	$1.13^{a} \pm 0.08$
Butrtylcholine esterase (U/g tissue)	$314.62^{a} \pm 7.89$	$551.49^{d} \pm 9.16$	$414.31^{b} \pm 25.15$	$479.83^{c} \pm 19.89$	$375.17^{b} \pm 20.59$
Malondialdehyde (nmol/g tissue)	$5.05^{a} \pm 0.28$	$14.94^{d} \pm 0.76$	$9.34^{b} \pm 0.52$	$11.49^{c} \pm 0.60$	$8.50^{b} \pm 0.36$
Catalase (μmol/g tissue)	$3.31^{a} \pm 0.29$	$6.10^{c} \pm 0.36$	$4.98^{b} \pm 0.30$	$5.46^{bc} \pm 0.39$	$4.63^{b} \pm 0.22$
Nitric oxide (nmol/g tissue)	$1.92^{a} \pm 0.24$	$3.59^{c} \pm 0.22$	$2.95^{bc} \pm 0.31$	$3.21^{c} \pm 0.32$	$2.21^{ab} \pm 0.25$
Reduced glutathione (μmol/g tissue)	$13.41^{c} \pm 0.79$	$9.84^{a} \pm 0.47$	$11.97^{bc} \pm 0.55$	$10.50^{ab} \pm 0.59$	$12.42^{c} \pm 0.54$
Superoxide dismutase (U/g tissue)	$7.38^{d} \pm 0.42$	$4.18^{a} \pm 0.34$	$6.05^{bc} \pm 0.29$	$5.47^{b} \pm 0.14$	6.81 cd ± 0.28
Brain weight (g)	$1.56^{a} \pm 0.04$	$1.54^{a} \pm 0.09$	$1.68^{a} \pm 0.06$	$1.68^{a} \pm 0.09$	$1.60^{a} \pm 0.07$
Brain weight (%)	$0.41^{a} \pm 0.02$	$0.48^{a} \pm 0.03$	$0.49^{a} \pm 0.02$	$0.46^{a} \pm 0.02$	$0.45^{a} \pm 0.04$

Note: In each row same letter means non-significant difference, while different letter means significant difference at 0.05 probability. The data are expressed as mean values ± standard error. Al, rats treated with aluminum chloride; M + Al, rats treated with microcapsules along with aluminum chloride; CY + AL, rats treated with control yoghurt along with aluminum chloride; FY + Al, rats treated with fortified yoghurt along with aluminum chloride. Brain weight (%) = (brain weight/final body weight × 100).

weight, AChE, BChE, and the oxidative marker of brain (hippocampus and cortex homogenate) are shown in Table 7. The results declared a significant increase in AChE and BChE values in the aluminum chloride group. Also, Kaizer et al. (2005) reported that aluminum neurotoxicity elevates the AChE activity. Additionally, Gulya et al. (1990) found that aluminum neurotoxicity can cause an imbalance in the level of neurotransmitters and increase inflammatory cytokines. So, aluminum neurotoxicity can contribute to the etiology of Alzheimer's disease (Xie et al., 2015). The long exposure to aluminum can cause the accumulation of aluminum in the body resulting in pro-oxidant activity via catalyzing the production of superoxide anion through the Fenton reaction (Ruiperez et al., 2012). In the present study, administration of aluminum chloride produced oxidative stress which reflected on the significant ($p \leq .05$) elevations of MDA and NO and the reduction in GSH and SOD in comparison to normal rats. Also, Auti and Kulkarni (2019) found that aluminum chloride elevated MDA and reduced GSH in rats.

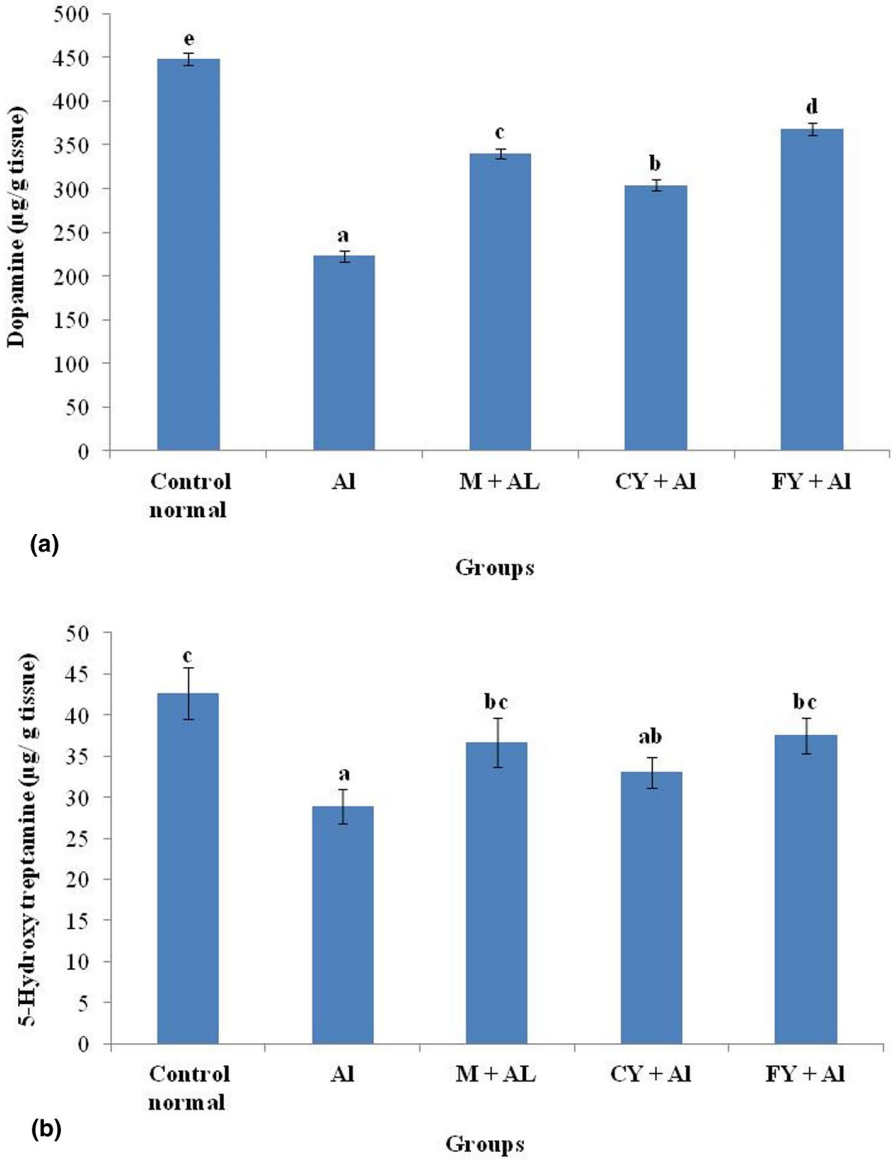

FIGURE 5 Dopamine of the different experimental groups (A), 5-hydroxytaptamine of the different experimental groups (B). On each par same letter means non-significant difference while different letter means significant difference at 0.05 probability. The data are expressed as mean values ± standard error. Al, rats treated with aluminum chloride; M + Al, rats treated with the triple omega microcapsules along with aluminum chloride; CY + AL, rats treated with control yoghurt along with aluminum chloride; FY + Al, rats treated with the fortified yoghurt along with aluminum chloride. ab, b, bc, c, d, and e < 0.05 versus control group

Oral administration with the triple omega microcapsules, the plain yoghurt or the fortified yoghurt suppressed the elevations of AChE, BChE, MDA, catalase activity, and NO. Rats treated with either the triple omega microcapsules or the fortified yoghurt recorded BChE, MDA, catalase activity, and NO values significantly lower than those of rats treated with the plain yoghurt. Additionally, rats treated with the fortified yoghurt recorded BChE, MDA, catalase activity, and NO values lower, although not statistically significant, than those of rats treated with the triple omega microcapsules. The fortified yoghurt was the most promising in reducing AChE and combating the reduction in GSH and SOD. There were not significant differences ($p > .05$) in the brain weight and the relative brain weight between the groups. Vitamins B content of the plain yoghurt might contribute to the reduction of the oxidative markers due to their antioxidant and anti-inflammatory effects. Thus, plain yoghurt may protect against the oxidative impairment of brain as confirmed by El-Abbadi et al. (2014). Also, lactic acid bacteria, fatty acids, and the peptides generated during the fermentation process of dairy product can positively affect the brain functions and prevent the cognitive decline as confirmed by Ano and Nakayama (2018). Omega-3 is considered prebiotics which modulate the gut microbiota, thus protect brain against oxidative stress and improve brain functions (Costantini et al., 2017). Khedr, (2017) found that wheat germ oil, the main source of omega-6, decreased brain AChE activity, MDA, and NO and increased brain GSH and SOD, due to its antioxidant power, in diabetic rats. Also, Kumar et al. (2019) found that

olive oil reduced AChE activity, MDA, and NO in rats treated with aluminum chloride. It was found that adding olive oil to food contributes to reducing MDA in the brain of geriatric rats, which contributes to protection from brain impairment (Mohamed et al., 2020). Liu et al. (2021) concluded that olive oil, as an essential component of the Mediterranean diet, protects against oxidative injury of brain and Alzheimer's disease in humans.

4 | CONCLUSIONS

Fortification of yoghurt with triple omega microcapsules significantly decreased the acidity and syneresis rate and increased viscosity. The sensory evaluation showed that the flavored yoghurt containing microcapsules had closer scores of sensory attributes to the plain yogurt. The flavored yoghurt containing microcapsules presented neuro-protective effect against the aluminum chloride-mediated neurotoxicity. It enhanced brain dopamine and 5-hydroxytriptamine, suppressed the elevation of brain acetylcholinesterase activity and attenuated the oxidative stress induced by aluminum chloride. The flavored yoghurt containing microcapsules improved the parameters mentioned above without causing severe weight loss as compared to the microcapsules alone.

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CONFLICTS OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS

Formal analysis; Methodology; Project administration; Writing-review & editing: Manal Ramadan. *Formal analysis; Methodology; Writing-original draft; Writing-review & editing*: Marwa El-Said. *Formal analysis; Methodology; Writing-original draft; Writing-review & editing*: Tamer El-Messery. *Formal analysis; Methodology; Writing-original draft; Writing-review & editing*: Rasha Salah Mohamed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the article.

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