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ORIGINAL PAPER

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PHYSICOCHEMICAL, ANTIOXIDANT, AND ORGANOLEPTIC CHARACTERISTICS OF CHEWING GUM ENRICHED WITH MICROENCAPSULATED *MELISSA OFFICINALIS* ESSENTIAL OIL USING ARABIC GUM AND KEFIRAN

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ABSTRACT

Background. Gum enriched with bioactive substances has the potential to be absorbed quickly through the mouth. The present research aims to model *Melissa officinalis* essential oil (MOEO) microcapsules as a health-functional ingredient to enhance the quality traits of chewing gum.

Materials and methods. Initially, evaluation assays were conducted on MOEO microcapsules. Subsequently, concentrations ranging from 0% to 6% were incorporated into chewing gum to investigate its physicochemical, antioxidant, antimicrobial and morphological properties.

Results. The findings demonstrated a maximum microencapsulation efficiency of 96.07%, with an embedding rate of 14.13% and a release rate ranging from 0% to 75% over 20 days. These outcomes were observed in the treated samples with a coating-to-essential oil (EO) ratio varying from 1:1 to 1:6. All microcapsules were confirmed through scanning electron microscopy (SEM) images. Antibacterial assays against *Streptococcus mutants* revealed that all microcapsules (at a concentration of 6400 µg/mL EO) exhibited antibacterial activity, and the diameters of the inhibition zones increased with higher concentrations. There was no statistically significant difference in ash and moisture content between chewing gum containing microcapsules and the control sample; however, higher levels of bioactive compounds were detected in the enriched samples. Significant antioxidant activity (IC_{50}, 31.00 mg/g) was observed in the gum sample containing 6% MOEO. All samples showed the largest inhibition zones against *Staphylococcus aureus*.

Conclusions. Overall, the results indicated that MOEO microcapsules had a significant effect on enhancing the quality of chewing gum, suggesting their potential as a suitable matrix.

Keywords: essential oil, chewing gum, *Melissa officinalis*, microcapsule, *Streptococcus mutans*

INTRODUCTION

Chewing gum is a chewy product created by combining a gum base or core with ingredients such as sweeteners, glucose syrup, preservatives, and functional compounds (Yingngam, 2022). It offers several oral health benefits, including removing food residues, reducing dry mouth, increasing biofilm pH, re-mineralizing tooth enamel, enhancing saliva secretion, and even serving as a medium for drug delivery. Additionally, chewing gum provides cognitive benefits such as stress reduction (Wessel et al., 2016). However, the production of chewing gum faces challenges, including limitations in sustained flavor release, regulatory

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restrictions, and customer preferences. Ideally, chewing gum would maintain its flavor even after extended chewing (Nouri et al., 2012).

Nowadays, various bioactive substances such as essential oils (EOs) are widely employed to produce medicinal gums with multiple functional attributes to meet the evolving needs of consumers (Mehta et al., 2022). *Melissa officinalis*, commonly known as lemon balm or balm mint, is a medicinal plant from the Lamiaceae family and Nepetoideae subfamily (Abdullatif et al., 2023). The main active constituents of *Melissa officinalis* essential oil (MOEO) include volatile substances (geranial, neral and geraniol), triterpenes (ursolic and oleanolic acids), phenolics (rosmarinic, caffeic and protocatechuic acids) and flavonoids (quercetin and rhamnocitrin) (Miraj et al., 2017).

Encapsulation is an emerging technology that involves coating EOs within a material, either polymeric or non-polymeric, to enable their controlled release under specific conditions. It is particularly used as a carrier in drug delivery and for the controlled release of preservatives and phytochemicals (Liu et al., 2017; Trevisan et al., 2022). MOEO has various health benefits, such as reducing inflammation, exhibiting antibacterial properties, improving skin health (treating eczema, acne, and psoriasis), treating Alzheimer's disease, anti-diabetic activity and decreasing high blood pressure due to its phytochemicals (Petriso et al., 2022).

There are diverse and unique techniques for encapsulating different bioactive ingredients, including spray drying, chilling and cooling, emulsification, fluidized bed coating, liposome entrapment and coacervation (Carvalho et al., 2023). Encapsulation produces microcapsules containing two main components, specifically the wall and core, which release their contents under specific conditions at controlled rates (Liu et al., 2017; Trevisan et al., 2022). The wall material of microcapsules must be carefully selected to ensure suitability for specific application purposes. The wall acts as the coating material and can be flexible, brittle, hard, or thin, facilitating controlled release at the required site under specific conditions (Al-Hamayda et al., 2023).

Arabic gum has been used to produce curcumin nanoparticles (Ai et al., 2023) and to encapsulate amino acids (Su et al., 2022). Polysaccharides, such as Arabic gum and kefiran, have been studied for their potential to improve the stability of EOs (Raksa et al., 2017). Several studies have investigated the utilization of bioactive components in chewing gum matrices (Konar et al., 2016; Miller et al., 2022; Qaziyani et al., 2019; Yingngam, 2022). For instance, MOEO has been studied as an antimicrobial agent in watermelon juice (Carvalho et al., 2023) and as a potential treatment for oral candidiasis (Serra et al., 2020).

However, there has been limited focus on understanding how these bioactive components affect the physiochemical, antioxidant and sensory traits of chewing gum along with its health benefits (Yingngam, 2022). The present research aims to model the effect of encapsulated MOEO on the physicochemical and antioxidant attributes of chewing gum.

MATERIALS AND METHODS

Materials

Melissa officinalis was purchased from Barij Company (Kashan, Iran), and gum base (white and non-acidic) was obtained from Zak Company. Sucrose, lecithin, glycerin, glucose, sorbitol, and inulin were procured from Merck Company (Germany). The bacterial strains, including *Streptococcus mutans* (*S. mutans*), *Staphylococcus aureus* (*S. aureus*), *Helicobacter pylori* (*H. pylori*), *Listeria monocytogenes* (*L. monocytogenes*) and *Escherichia coli* (*E. coli*) were acquired from the Iranian Research Organization of Science and Technology. The necessary chemicals and culture media were obtained from Merck (Germany) and Q-Lab (Canada), respectively.

Manufacture of microcapsules containing MOEO

2.5 g of kefiran powder and 2.5 g of Arabic gum were weighed and dissolved separately in 100 mL of water at 45°C for 1 hour under constant stirring at approximately 100 rpm. In the next step, 0.4% (w/v) Tween 80 was added to the kefir solution as a surfactant, and the mixture was stirred continuously in a homogenizer (Universal 320, Hettich, Tuttlingen, Germany) at 6000 rpm for 10 minutes. The Arabic gum solution was then added.

In the final step, different ratios of coating extract (1:1, 1:2, 1:3, 1:4, 1:5, and 1:6) were prepared for the treatments T_1, T_2, T_3, T_4, T_5, and T_6. The mixture was

allowed to naturally cool to approximately 30°C, after which the temperature was reduced to 10°C. The samples were then dried using a freeze dryer, and the resulting microcapsules were stored at 4°C until the assays were performed. The samples were labeled as control and T_1 to T_6 (Raksa et al., 2017).

Performance evaluation tests of microcapsule

Encapsulation efficiency and loading capacity

The MOEO content was measured using an ultraviolet−visible (UV−vis) spectrometer at 290 nm (Thermo Scientific, Madison, WI, USA), and a standard calibration curve was plotted against the various concentrations (Liu et al., 2017).

Release rate of MOEO

Simulated gastrointestinal fluids (HCl 0.1 M, with pH 2.0 adjusted by NaOH) were applied to investigate the release of MOEO samples from hydrogel beads. 1 g of microcapsules was mixed with 2 mL of gastric fluid and incubated at 37°C under constant for 3 hours. Afterwards, the microcapsules were filtered and placed in simulated intestinal fluid (phosphate buffer with pH 7.5) at 37°C for approximately 3 hours. The released percentage of MOEO was calculated using Eq. 1 (Najafi-Soulari et al., 2016):

$$\text{Release EO} = (M_c/M_e) \times 100 \quad (1)$$

where M_c and M_e represent the content of released MOEO and the encapsulated forms in hydrogel beads, respectively.

Antibacterial assay

A half-McFarland bacterial suspension was prepared on a Mueller-Hinton agar medium in a uniform layer to measure the antibacterial function of *S. mutans* using the well diffusion technique (Carvalho et al., 2023).

Chewing gum formulation

500 g of *Pistacia atlantica* gum base was added to 500 mL of water and boiled for 3 hours until the gum transformed into a chewable resin. The resulting resin was then placed in a stainless-steel laboratory container and heated in a water bath at 60°C. The other ingredients for the chewing gum formulation, including 68% sweetener, 0.5% lecithin, 0.5% glycerin, 1% inulin, and approximately 30% gum base, were then added. For the control and other samples with microencapsulated MOEO, the following percentages of MOEO were used: 0%, 1%, 2%, 3%, 4%, 5%, and 6% (labeled as CG_{C0}, CG_{T1}, CG_{T2}, CG_{T3}, CG_{T4}, CG_{T5}, and CG_{T6}), respectively. The control sample contained approximately 29% gum base (Qaziyani et al., 2019).

Assays for chewing gum containing MOEO microcapsules

Determination of Ash and Moisture Contents

The ash content and moisture content of the samples were measured according to standard guidelines (Kaveh et al., 2023).

Antioxidant activity assay

The antioxidant activity of chewing gums, including those containing MOEO, was evaluated using the DPPH free radical scavenging assay. A stock solution of 0.1 mM DPPH in a water/methanol (70:30) mixture was first prepared. Then, 1 mL of MOEO was added to 3 mL of 0.1 M DPPH solution. The mixture was thoroughly vortexed and left in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm wavelength using a spectrophotometer (Abdellatif et al., 2023).

Antibacterial activity of chewing gum against pathogen bacteria

The antimicrobial activity of the chewing gum against pathogenic bacteria (*H. pylori* ATCC 43629, *S. aureus* ATCC 25923, *L. monocytogenes* ATCC 19115 and *E. coli* ATCC 10536) was assessed.

Morphological assay of chewing gum

The microstructure of chewing gum containing microcapsules was examined using scanning electron microscopy (SEM) (phenom, Poland) with a 25 kV acceleration voltage (Palabiyik et al., 2018).

Statistical analysis

All experiments in this study were performed in triplicate, and the results are presented as means ±standard deviation (SD). The data were first assessed for normality and variance. Statistical analysis was performed using one-way ANOVA, and the means were compared using SPSS statistics V21.0 (IBM, Armonk, USA).

RESULTS AND DISCUSSION

Encapsulation efficiency, encapsulated MOEO, and release rate

The results demonstrated that a higher concentration of MOEO increased the encapsulation efficiency, which was consistent with the increase in the encapsulated EO content. Encapsulation efficiency refers to the ratio of MOEO present in the microcapsules to the total amount of oil added during production, which increases with a higher concentration of MOEO. Increasing the MOEO concentration from 1% (T_1) to 6% (T_6) resulted in an improvement in encapsulation efficiency, from 93.26% to 96.07%, and in the amount of encapsulated component, which increased from 10.04% to 14.13 % (Table 1).

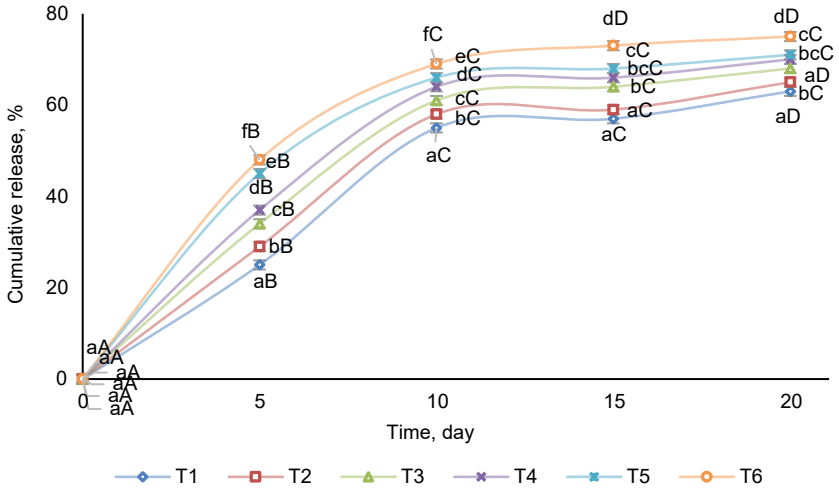
In a previous study, encapsulation of *Melissa officinalis* aqueous extract and calcium alginate hydrogel was examined (Najafi-Soulari et al., 2016). The most efficient encapsulation of *Melissa officinalis* was achieved with an 84.1% sodium alginate solution, 2.0% calcium chloride, and a specific extract concentration. The results demonstrated that a mixture of polysaccharides improved encapsulation efficiency (Mansour et al., 2019). Encapsulation efficiency for MOEO was detected as 90% using why protein isolate/sodium caseinate and 99.4% with microencapsulation in a maltodextrin polymer matrix (Bisht et al., 2022; Karimi Sani et al., 2020).

Figure 1 illustrates the cumulative release of MOEO from different samples. The cumulative releases for samples T_1 to T_6 were: 62%, 65%, 67%, 70%, 71%, and 74%, respectively, after 20 days. The maximum

Table 1. Encapsulation efficiency and surface oil of microcapsules for MOEO

Treatment	T_1	T_2	T_3	T_4	T_5	T_6
Efficiency Encapsulate (%)	93.26 ± 0.32^{dA}	93.93 ± 0.29^{dA}	94.21 ± 0.44^{cA}	94.94 ± 0.09^{bcA}	95.48 ± 0.51^{abA}	96.07 ± 0.11^{aA}
Encapsulated MOEO (%)	10.04 ± 0.29^{dB}	$10.27^{dB} \pm 0.30$	$11.48^{cB} \pm 0.08$	12.50 ± 0.20^{bB}	13.82 ± 0.27^{aB}	14.13 ± 0.80^{aB}

Ratios for coating to extract (1:1, 1:2, 1:3, 1:4, 1:5, and 1:6 in treatments of T_1, T_2, T_3, T_4, T_5 and T_6).
Different lowercase letters indicate significant differences in each row ($p < 0.05$) and distinct uppercase letters indicate significant differences in each column ($p < 0.05$).



Ratios for coating to essential oil (1:1, 1:2, 1:3, 1:4, 1:5, and 1:6 in treatments of T_1, T_2, T_3, T_4, T_5, and T_6). Different lowercase letters indicate significant differences between samples, and capital letters indicate significant differences between various days ($p < 0.05$).

Fig. 1. Cumulative release curve of microcapsules for MOEO during 20 days

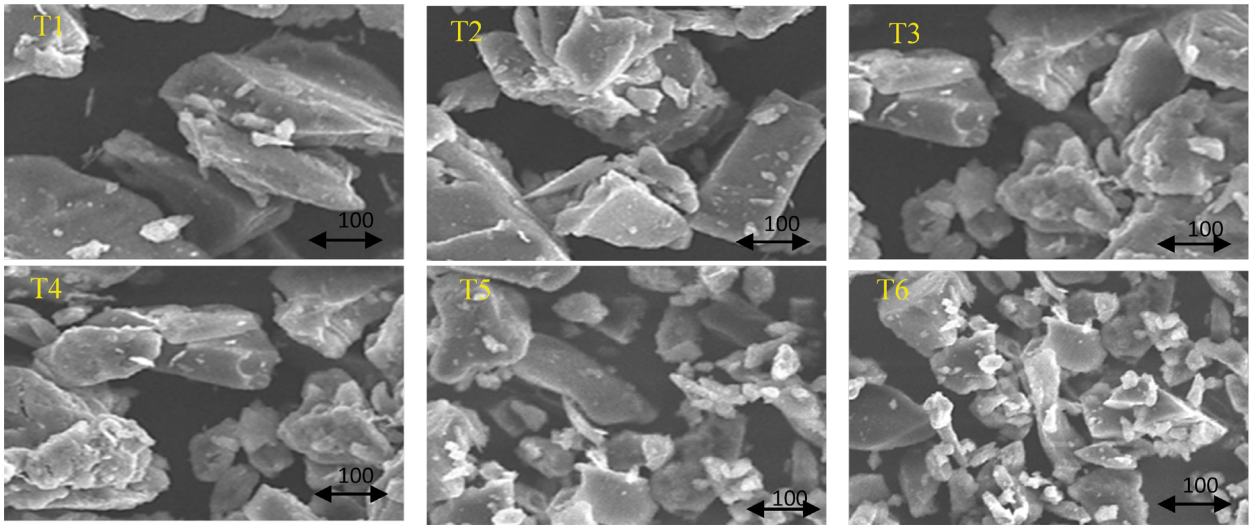


Fig. 2. Scanning electron microscopy images of microcapsules for MOEO. Ratios for coating to extract (1:1, 1:2, 1:3, 1:4, 1:5, and 1:6 in treatments of T_1, T_2, T_3, T_4, T_5, and also T_6)

release was observed in T_6, while the minimum was in T_1, with a significant difference between the samples ($p < 0.05$). The release was rapid during the first 10 days, after which the rate gradually decreased. The cumulative release of cinnamon EO/xanthan gum/chitosan microcapsules showed that 60% of the EO was released at 37°C, over 400 hours, which was mainly attributed to easier diffusion near the inner surface of the shells and reduced concentration differences between the inner and outer environments of the composite microcapsules (Li et al., 2022).

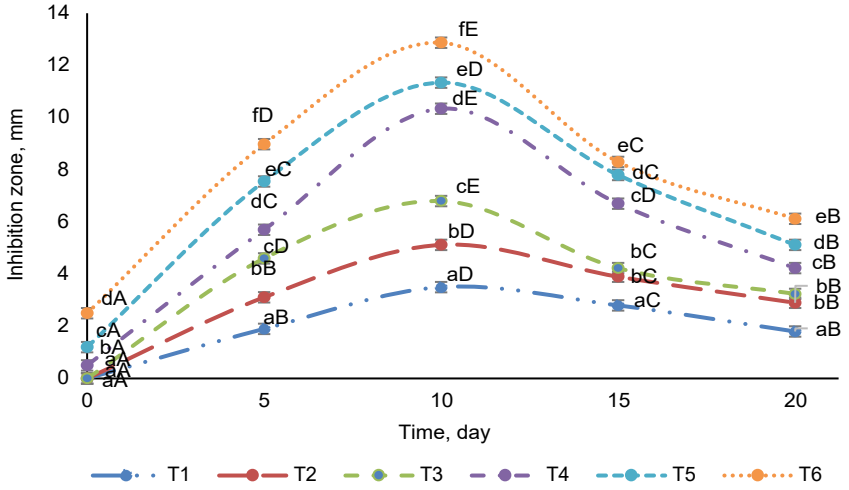
Morphology of microcapsules

According to the images obtained for the samples (Fig. 2), the level of MOEO in microcapsules increases from T_1 to T_6, and the particle size distribution becomes more uniform with a smoother surface. No aggregation is observed in any of the samples. Arabic gum, an anionic polysaccharide, is used as a wall-coating material in the microencapsulation process. It contains carboxylate groups with negative charges and a zeta potential, which enhances electrostatic repulsion forces between the microcapsule particles, thereby reducing aggregation (Raksa et al., 2018).

Food materials create surface areas that can protect entrapped molecules from exposure to heat and oxygen (Palabiyik et al., 2018). Microcapsules containing Arabic gum and kefiran exhibited crystalline polymorphic structures, with a greater MOEO content leading to a smoother surface (Bisht et al., 2022). Higher hydrogen ion concentrations promote the rearrangement of protonated Arabic gum and kefiran-free chains, resulting in the formation of semi-spherical structures and homogeneous aggregation of microcapsules (Raksa et al., 2017).

Antimicrobial activity against *S. mutans*

The highest sensitivity against *S. mutans* was observed in T_6 and T_5, where higher MOEO levels demonstrated significant antibacterial activity against the target bacteria, with inhibition zones of 14 mm and 13.2 mm, respectively, showing a significant difference ($p < 0.05$) (Fig. 3). The greatest antimicrobial inhibition zone was observed in all samples during the first 10 days, after which the inhibition zone decreased. These changes in the inhibition zone were due to the release rates of MOEO over time, with the highest release occurring on the 10th day. The strain of *S. mutans* which has altered carbohydrate metabolism and a strong ability to produce acids (including lactic, acetic and formic acids) is responsible for causing major cavities (Carvalho et al., 2023). The action mechanism of EOs and plant extracts is associated with their chemical composition and antibacterial activity; however, they do not



Different lowercase letters indicate significant differences between samples, and capital letters indicate significant differences between various days ($p < 0.05$).

Fig. 3. The growth inhibition zone of microcapsules for MOEO during 20 days. Ratios for coating to extract (1:1, 1:2, 1:3, 1:4, 1:5, and 1:6 in treatments of T_1, T_2, T_3, T_4, T_5, and T_6)

all interact in the same way. Nevertheless, their effects on the cell membrane of microorganisms have been confirmed in most studies. The lipophilic nature of extracts and EOs is clearly linked to their antibacterial functions (Abdellatif et al., 2023). This enables them to penetrate the lipids components of cell wall structure, enhancing membrane permeability, disrupting all vital processes dependent on membranes (including electron transport and ion release), and ultimately causing cell death (Mehta et al., 2022). Phenolic compounds affect membrane structure, which can not only attack the cell wall and affect fluidity and permeability but also disrupt the functions of the membrane framework, thereby inhibiting microbial growth (Miraj et al., 2017). The antibacterial effect of MO methanolic extract against *S. mutans* increased with higher extract concentrations, showing enhanced antibacterial activity (Carvalho et al., 2023). In line with the present results, the effect of sugar-free chewing gum reduced *S. mutans* bacteria in children's mouths, thus preventing tooth decay (Palabiyik et al., 2018).

Physicochemical and antioxidant traits of chewing gum

The results in Table 2 show that moisture content ranged between 6.50% and 7.65% and CG_{C0} exhibited a significantly lower level compared to higher levels in the treated samples. The higher amount of EO significantly promoted moisture content in gum samples containing microcapsules compared to the control, which was attributed to their polysaccharide coating ($p < 0.05$). According to the results, incorporating microcapsules up to 3% did not affect the ash and mineral content of the chewing gum samples. However, these levels increased significantly when the concentration was raised to 6%. The results demonstrated that the lowest antioxidant activity was observed in the control sample (47.13 mg/g), while CGT_6 (31.00 mg/g), which contained more MOEO, exhibited the highest activity (Table 2).

Chewing gum is a highly stable product due to its low moisture content and lower reactivity compared to other oral ingredients (Kaveh et al., 2023). Arabic gum in food products helps retain moisture, and freeze-drying is the most effective technique for producing frameworks that serve as agents for encapsulating bioactive substances (Raksa et al., 2017). Studies have reported that the moisture content of chewing gum ranges from 2% to 8% (Yakoubi et al., 2021; Yingngam, 2022), which aligns with the findings of the present research. The inhibitory power of different samples depends significantly on the number and position of hydroxyl groups and the molecular weight

Table 2. Physicochemical characteristics for microcapsules of chewing gum

Treatment	Moisture, %	Ash, %	Antioxidant activity: IC50, mg/g
CG_{C0}	6.50 ±0.19^{b}	0.89 ±0.01^{b}	47.13 ±0.50^{c}
CG_{T1}	7.64 ±0.05^{a}	0.89 ±0.01^{b}	34.11 ±0.12^{b}
CG_{T2}	7.62 ±0.41^{a}	0.89 ±0.01^{b}	34.0 ±0.07^{b}
CG_{T3}	7.63 ±0.13^{a}	0.90 ±0.04^{b}	33.05 ±0.29^{b}
CG_{T4}	7.64 ±0.33^{a}	0.91 ±0.01^{ab}	32.90 ±0.10^{b}
CG_{T5}	7.65 ±0.24^{a}	0.92 ±0.01^{a}	32.40 ±0.11^{b}
CG_{T6}	7.63 ±0.11^{a}	0.92 ±0.01^{a}	31.00 ±0.29^{a}

Samples with microencapsulated MOEO (0%, 1%, 2%, 3%, 4%, 5%, and 6%, labeled as CG_{C0}, CG_{T1}, CG_{T2}, CG_{T3}, CG_{T4}, CG_{T5} and CG_{T6}). Different lowercase letters indicate significant differences ($p < 0.05$).

of phenolic compounds (Yakoubi et al., 2021). MOEO exhibited a strong inhibitory effect (94.31 ±0.082%) and significant antioxidant potential against DPPH free radicals (IC_{50} = 2.78 µg/mL), which was positively correlated with its high rosmarinic acid content (Karimi Sani et al., 2020).

Antimicrobial attributes

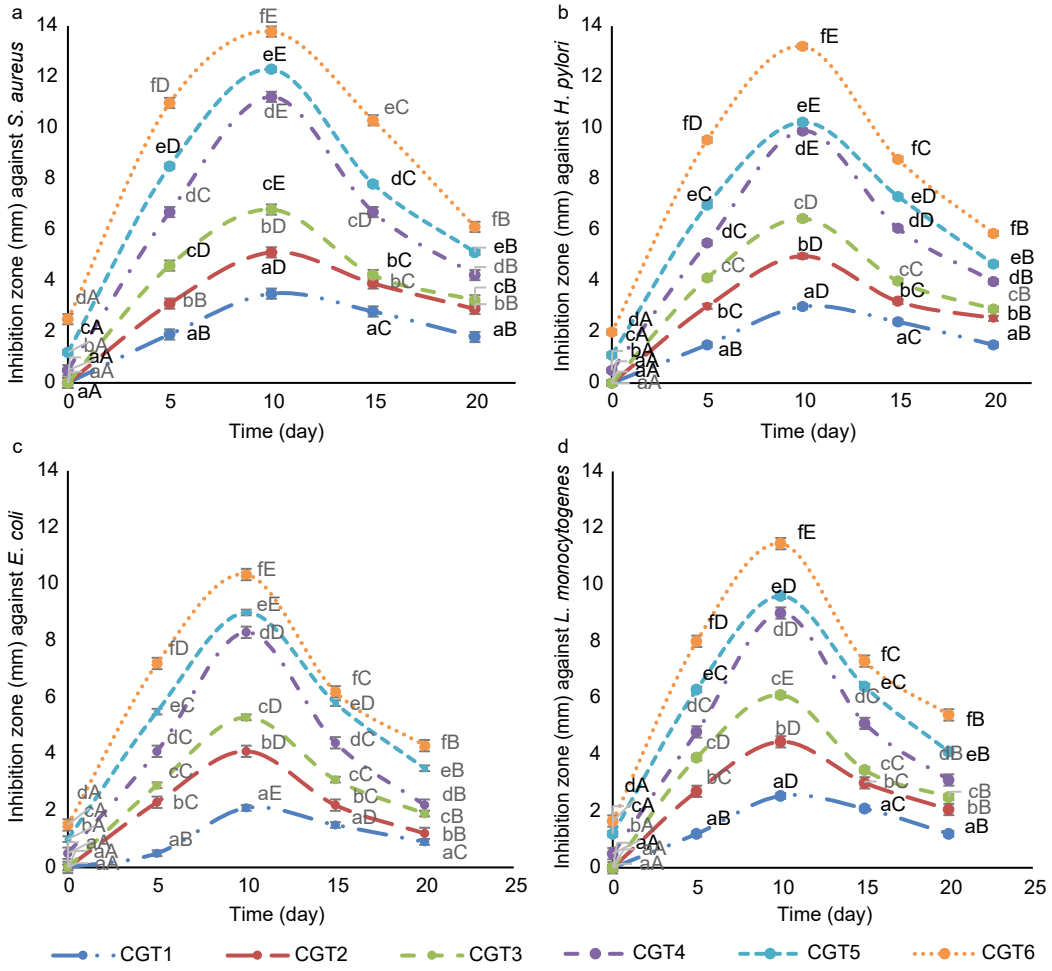
The results highlighted the antimicrobial efficacy of the microcapsule samples against harmful digestive system bacteria over time (Fig. 4). Among all samples, the highest level of inhibition was observed against *S. aureus*, followed by *H. pylori*, *L. monocytogenes* and *E. coli*, respectively ($p < 0.05$). Notably, the CG_{T6} sample exhibited the largest inhibition zone, significantly outperforming CG_{T5} and CG_{T4} ($p < 0.05$). During the initial 10 days of storage, there was a progressive increase in inhibition, signifying heightened antimicrobial effectiveness. However, this trend reversed beyond this period, and the inhibition level began to decline. This decrease in antimicrobial activity corresponded with the diminishing release of MOEO after the 10-day mark. The reduction in release rate beyond the 10th day directly contributed to the observed decline in antimicrobial efficacy.

The initial potency followed by a decline underscores the importance of sustained release strategies for maintaining prolonged antimicrobial benefits in such formulations. MOEO and *Pistacia atlantica* can also be considered influential agents against *H. pylori* and peptic ulcers, with several studies supporting the effectiveness of Saqqez against *H. pylori* and peptic ulcers (Carvalho et al., 2023). The antimicrobial activity of *Pistacia atlantica* has been proven against strains of *E. coli*, *S. aureus* and *Streptococcus pyogenes*, due to its polyphenol compounds (Kaveh et al., 2023).

MOEO has demonstrated strong antimicrobial activity against *L. monocytogenes* in watermelon juice (Carvalho et al., 2023), *E. coli* and *S. aureus* (Miller et al., 2022) and *Candida spp*. (Miraj et al., 2017). Among these bacteria, *S. aureus*, a gram-positive bacterium, exhibited the highest sensitivity, while *E. coli*, a gram-negative bacterium, showed the lowest sensitivity to the antimicrobial features. This difference is due to *S. aureus'* monolayered structure, primarily composed of peptidoglycan, and *E. coli's* more complex structure, which includes lipopolysaccharides and also an inner phospholipid membrane (Abdellatif et al., 2023).

Morphology of chewing gum containing microcapsules

Figure 5 depicts the chewing gum containing EO microcapsules, which are evenly dispersed and encapsulated within the chewing gum matrix. The surface of chewing gum appears smooth, with tiny spherical microcapsules present and no visible EO droplets. However, the morphological analysis indicates distinct visual differences in the microstructure. The surface of the chewing gum exhibited widened and non-homogeneous characteristics, with some pores of varying sizes observed on the surface.



Different lowercase letters indicate significant differences between samples and capital letters indicate significant differences between various days ($p < 0.05$).

Fig. 4. The growth inhibition zone of microcapsules for MOEO during 20 days against (a) *S. aureus*, (b) *H. pylori*, (c) *L. monocytogenes* and (d) *E. coli*, Samples with Microencapsulated MOEO (0%, 1%, 2%, 3%, 4%, 5% and 6% as CG_{C0}, CG_{T1}, CG_{T2}, CG_{T3}, CG_{T4}, CG_{T5} and CG_{T6})

The stability of larger microcapsules can be advantageous in ensuring the longevity and effectiveness of encapsulated EOs within chewing gum (Palabiyik et al., 2018). The presence of menthol microcapsules in gum structures has been shown to affect the morphology of microcapsules (Abdellatif et al., 2023), which corroborates the findings of the present research.

CONCLUSION

In the current study, Arabic gum and kefiran, being water-soluble and possessing adequate emulsifying functions, were used to prepare microcapsules containing MOEO, thereby promoting efficacy. The best stability was observed in T_4 (ratio 1:3), resulting in reduced aggregation and improved encapsulation efficiency, which was confirmed by SEM images. The antibacterial assay revealed that all microcapsules exhibited this function. Antioxidant activity improved with the addition of microcapsules, with a more pronounced effect at higher MOEO concentrations. Maximum inhibition was observed against *S. aureus*, *H. pylori*, *L. monocytogenes* and *E. coli*, respectively. Despite certain limitations, such as the inability to

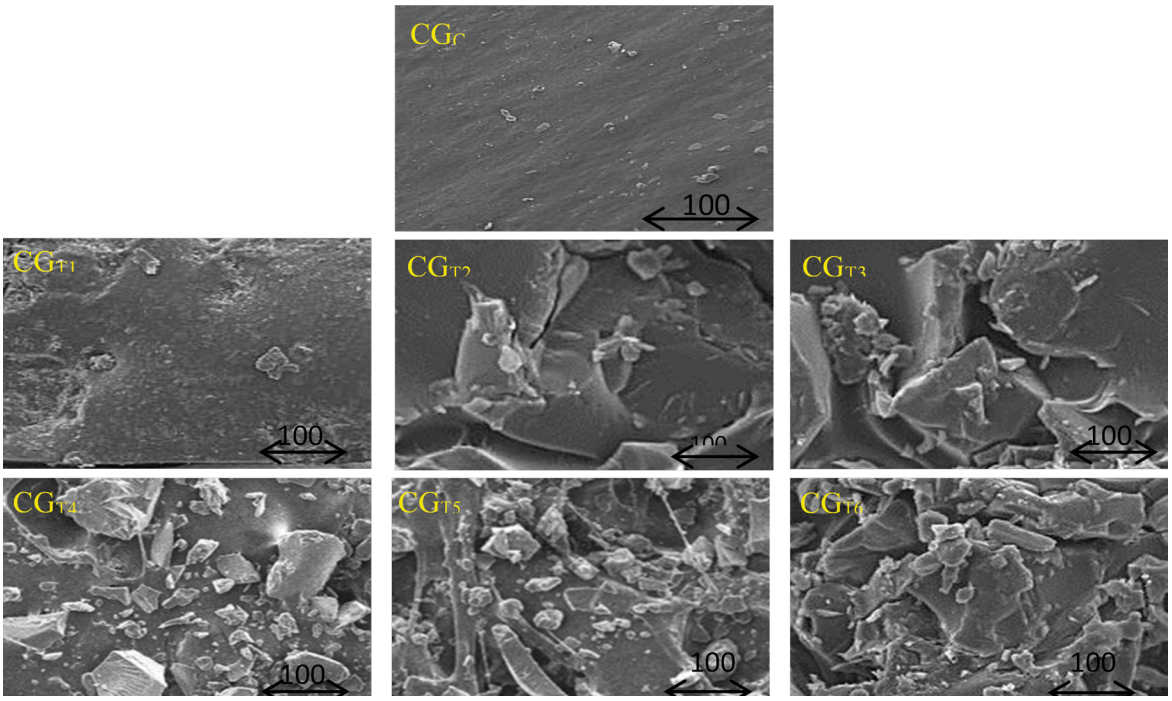


Fig. 5. Scanning electron microscopy images of microcapsules and chewing gum containing microcapsules. samples with microencapsulated MOEO (0%, 1%, 2%, 3%, 4%, 5%, and 6% as CG_{C0}, CG_{T1}, CG_{T2}, CG_{T3}, CG_{T4}, CG_{T5}, and CG_{T6})

conduct long-term tests on animals or humans, the developed gum demonstrates the necessary potential for industrial production and public use.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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