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Effect of freeze-drying and oven-drying on the physicochemical and morphological properties of marjoram essential oil beads produced by ionic gelation

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This study explored the impact of different drying methods – freeze-drying (FD) and oven-drying (OD) – on the physicochemical properties of marjoram essential oil (MEO) beads produced by ionic gelation. Microencapsulation was performed by extrusion–dripping of emulsions containing 2 g/100 g MEO, 1.25 g/100 mL sodium alginate and 1.25 g/100 mL whey protein isolate into a 0.175 mol L^{-1} $CaCl_2$ gelling bath. The beads were dried either by freeze-drying (FD) for 24 h or by hot-air oven drying (OD) at 60 °C for 24 h. After drying, the beads were characterized in terms of encapsulation efficiency, chemical composition by GC-MS and GC-FID, moisture content, and thermal degradation by thermogravimetric analysis (TGA). The beads' morphology was evaluated by scanning electron microscopy (SEM). FD beads retained a higher MEO content (51.25%) compared to OD beads (33.75%) ($p < 0.05$). Morphological analysis indicated a brittle structure and larger pores in FD beads, whereas OD beads exhibited a denser and less porous surface. Moreover, FD beads showed higher thermal stability than OD beads. The study concludes that the choice of drying technique plays a crucial role in the beads' physicochemical and morphological properties. Overall, the results demonstrate that the drying method plays a crucial role in the physicochemical and morphological properties of the beads, with freeze-drying being more effective in preserving essential oil content and thermal stability.

Sustainability spotlight

This work contributes to expanding the application scope of sustainable strategies in the food industry, by evaluating different drying methods to produce particles of marjoram essential oil microencapsulated by ionic gelation. The process employs natural biopolymers and mild, solvent-free conditions, minimizing energy use and environmental impact. The resulting ingredient is a natural renewable bio-based additive with potential preservative properties, offering a sustainable alternative to synthetic food additives. The research aligns with the United Nations Sustainable Development Goals, particularly SDG 12 (Responsible Consumption and Production), since it promotes the development of sustainable processes for obtaining natural additives with low environmental impact, and SDG 9 (Industry, Innovation and Infrastructure), as it encourages the adoption of innovative and cleaner technologies for the food, pharmaceutical, and cosmetics industries.

1 Introduction

The investigation of effective strategies to enhance food quality has long been an area of intense research. Essential oils are liquid extracts obtained from plant raw materials and are rich in bioactive and aromatic compounds, mostly volatile constituents such as terpenoids, phenolic compounds and aldehydes, which confer their characteristic aroma and, in some cases, antimicrobial and antioxidant activities. Owing to these properties, essential oils have attracted increasing interest for application in food products to improve flavor and extend shelf-life.[1,2] Marjoram (*Origanum majorana* L., family Lamiaceae) essential oil is widely utilized as a condiment and is valued for its distinctive aroma and antimicrobial properties.[3,4]

Microencapsulation can be employed as an effective strategy to protect essential oil constituents against losses, degradation, and volatilization. This technique involves entrapping a target compound within a wall material in order to enhance its stability and extend shelf life.[5,6] For heat-sensitive compounds, such as those present in essential oils, ionic gelation is one of the most suitable microencapsulation techniques, as it is performed under mild conditions and at ambient temperature.

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Ionic gelation consists of entrapping a bioactive substance within a three-dimensional polymeric network, resulting in the formation of gel particles commonly referred to as beads. These beads are produced by the crosslinking of anionic polymers (such as sodium alginate, pectin and others) with di- or polyvalent cations, such as calcium ions.[7]

Beads produced by ionic gelation typically contain a high moisture content, which make them susceptible to deterioration, depending on the storage conditions. In this sense, drying can be used as an additional step to improve ingredient stability, as it reduces microbial growth and enzymatic activity.[8–10] Freeze drying and oven drying are two commonly used methods for dehydrating food products and ingredients. Freeze drying, also referred to as lyophilization, involves freezing the product followed by moisture removal under vacuum by sublimation.[11] In contrast, oven drying relies on the application of heat to evaporate water from the product.

Given that essential oils contain heat-sensitive compounds, it is crucial to evaluate the effects of these drying techniques on the properties of essential oil beads. Several studies have investigated the effects of drying methods on the stability and thermal behavior of microencapsulated systems.[11–13] Nevertheless, comparative studies specifically addressing the effects of oven-drying and freeze-drying on beads produced by ionic gelation remain scarce in the literature, highlighting the relevance of the present investigation.

This study aimed to evaluate and compare the effects of freeze drying and oven drying on the physicochemical properties of marjoram essential oil beads produced by ionic gelation. The properties assessed after drying included encapsulation efficiency, chemical composition, moisture content, thermal degradation, and morphological characteristics.

2 Materials and methods

2.1 Chemicals

Commercial marjoram essential oil, hydrodistilled from the leaves of Egyptian marjoram (*O. majorana*), was acquired from Ferquima® (Vargem Grande Paulista, São Paulo, Brazil). Sodium alginate (Gastronomy Lab®, Brasília, DF, Brazil), whey protein isolate (WPI) (Alibra®, Campinas, São Paulo, Brazil) and dihydrated calcium chloride P.A. (Vetec Química Fina®, Duque de Caxias, Rio de Janeiro, Brasil) were used, respectively, as an encapsulating polymer, as an emulsifier, and in the formulation of the cationic bath. Methyl octanoate (99,8% purity), used as the internal standard, and the authentic standards of sabinene, *p*-cymene, limonene, γ-terpinene, *cis* and *trans*-sabinene hydrate, and terpinen-4-ol, as well as a mixture of *n*-alkanes for LRI calculation, were provided by Sigma Aldrich® (Darmstadt, Germany).

2.2 Microencapsulation by ionic gelation

Emulsions were prepared by adding the oil phase dropwise to the aqueous polymer solution under constant mechanical stirring at 1800 rpm for 5 min using an Ultra-Turrax (IKA T8 Basic, Wilmington, USA). This procedure was selected based on preliminary trials and previous work,[14] ensuring homogeneous dispersion of the oil droplets. The resulting emulsion, composed of 2 g/100 g of marjoram essential oil, 1.25 g/100 mL sodium alginate, and 1.25 g/100 mL whey protein isolate (WPI), was then subjected to ionic gelation *via* extrusion–dripping using a 22G gauge syringe (0.70 mm diameter and 25 mm length) coupled to a vacuum system. Beads were formed in a 0.175 mol L^{-1} $CaCl_2$ gelling bath and remained under stirring for 30 min, following a protocol established in a previous study.[14]

2.3 Freeze-drying

Freeze-dried samples (FD) were produced by first freezing the wet beads at −17 °C in a freezer and then placing them into a benchtop freeze-dryer (Liobras, L101, Brazil) at −40 °C for 24 h. A freezing temperature of −17 °C was selected to ensure complete solidification of the bead matrix prior to freeze-drying. The drying was conducted at −40 °C under vacuum for 24 hours, aiming to preserve the integrity of heat-sensitive volatile compounds in the essential oil. Beads were kept in glass flasks stored in desiccators containing silica gel for further analysis.

2.4 Oven-drying

Oven-dried samples (OD) were prepared by drying the wet beads in a hot air circulation oven (Memmert, USA), at 60 °C for 24 h. This temperature was selected as a moderate condition to promote efficient moisture removal while minimizing excessive deformation, as suggested by Vargas *et al.* (2018).[15] Beads were kept in glass flasks stored in desiccators containing silica gel for further analysis.

2.5 Encapsulation efficiency

The marjoram essential oil content was determined by extracting the essential oil from the beads by hydrodistillation, after prior dissolution in a sodium citrate solution (10 g/100 mL), as previously described by Mazza *et al.*[14]. The volume of essential oil recovered was converted to mass using a density factor of 0.893 g mL^{-1}.

Encapsulation efficiency (EE) was calculated according to eqn (1):[16]

$$\text{EE }(\%) = \left[\frac{\text{MEO content in beads (mg)}}{\text{total MEO added to formulation (mg)}}\right] \times 100 \quad (1)$$

The total MEO added refers to the theoretical amount incorporated during emulsification, while the MEO content in beads was determined experimentally after encapsulation.

2.6 Chemical characterization

The compositions of both free marjoram essential oil and the essential oil recovered from the beads after hydrodistillation were analyzed by gas chromatography with mass spectrometry (GC-MS) and flame ionization detection (GC-FID), as described by Mazza *et al.*[14] GC-MS analyses were carried out using an Agilent 7890A gas chromatograph (Agilent, Delaware, USA) equipped with an Agilent 7693B automatic sampler and a 5975C mass selective detector. An Agilent HP-5MS capillary column (5%-phenyl-95%-methylsilicone, 30 m × 250 μm × 0.25 μm) was used, with helium as carrier gas (1.0 mL min^{-1}). The GC injector was kept at 250 °C. The mass detector was set to electron ionization (70 eV) in scan mode (3.15 scans per s), 40–350 u. The ionization source was operated at 200 °C, the analyzer at 150 °C and the transfer line at 260 °C.

The GC-FID analyses were run in an Agilent 7890B equipped with an Agilent 7693B automatic sampler and a FID detector, kept at 280 °C. The capillary column and operational parameters were the same as described for GC-MS, except for the carrier gas, which was hydrogen, at 1.5 mL min^{-1}. The samples were injected in triplicate. An internal standard (methyl octanoate, 408 μg mL^{-1}) was added to the samples and used for quantitation. Absolute areas from the FID signal were corrected with theoretical relative response factors.[17] The results were expressed as mg of the constituent per g of essential oil.

2.7 Morphology

The internal and external morphologies of the beads were evaluated by scanning electron microscopy (SEM). To reveal the inner bead, freeze-dried and oven-dried samples were cut with a craft knife. Prior to SEM imaging, samples were sputter-coated with a thin layer of gold using gold wire. Beads were observed in a Tescan Vega 3 (Tescan®, Kohoutovice, Czech Republic), operated at an accelerating voltage of 15 kV, under high-vacuum conditions.

2.8 Size and circularity

Bead size, encompassing both area (in mm^2) and diameter (in mm), along with shape (circularity), was assessed using the digital image processing tool ImageJ.[18] Feret's diameter was employed for evaluating bead sizes and for investigating beads' surface area, a measure obtained from a projection around the bead using the average diameter.[19] The wet bead circularity was determined using eqn (2), according to the ImageJ User Guide, from images of at least 100 beads captured using a digital camera.

$$\text{Circularity} = 4\pi r\ (\text{area/perimeter}) \qquad (2)$$

2.9 Moisture content

Moisture content of the freeze-dried and oven-dried beads was determined according to the 934.06 AOAC method[20] with adaptations for hydrogel-based samples. The beads were placed in a vacuum oven at 70 °C to remove moisture. After an initial drying period of 6 hours, samples were weighed at 1 hour intervals until constant mass was achieved (Δm < 0.001 g). Moisture content was expressed in percentage of the initial sample mass before drying (moisture g/100 g beads).

2.10 Thermal stability analysis (TGA)

Thermal stability of the dried beads was determined in a DiscoveryTM TGA 55 thermogravimetric analyzer (TA Instruments/Waters Corporation, USA). Samples (5.0 mg) were heated from 25 °C to 800 °C (10 °C min^{-1}) under nitrogen gas flow (30 mL min^{-1}). The results were expressed as T_{onset}, which is the temperature that indicates the onset of sample thermal degradation.

2.11 Statistical analysis

Statistical analyses were performed using Statistica 7.0 software (Statsoft Inc. 2004, Tulsa, OK, U.S). Comparisons were established from duplicate or triplicate results, with all data presented as means and standard deviations. Normality and homogeneity tests were conducted on the data, and statistical significance was considered at $p < 0.05$. T-test analysis was performed to assess differences in moisture content and thermal stability. Additionally, encapsulation efficiency, size and circularity results underwent analysis of variance (ANOVA). Statistical significance in chemical composition was evaluated with a T-test or one-way ANOVA followed by Tukey's pos-test for normal distribution data, and a Kruskal–Wallis test for non-parametric data, whenever appropriate.

3 Results and discussion

3.1 Chemical characterization

The chemical composition of marjoram essential oil (MEO) and the essential oils recovered from oven-dried (OD) and freeze-dried beads (FD) is shown in Fig. 1. Although the drying methods did not alter the qualitative profile of MEO, they promoted significant changes in the concentration of some compounds (Table 1).

The major compounds identified in the free marjoram essential oil were *trans*-sabinene-hydrate (21.8%) and terpinen-4-ol (21.1%), followed by γ-terpinene (12.0%) and sabinene (8.3%). These results are consistent with the literature, although the relative concentrations of these compounds may vary depending on the botanical origin of the essential oil. Moreover, marjoram essential oil is known to present at least two chemotypes: alcoholic and phenolic monoterpenoids. The absence of phenolic compounds in the oil used in the present study indicates that it belongs to the monoterpenic alcohol one.[21–25]

As shown in Table 1, noticeable variations in compound concentrations (mg g^{-1}) were observed among the three samples. Some constituents exhibited increased concentrations in OD or FD beads compared to MEO, whereas others showed significant reductions. Notably, the main compounds of free MEO – *trans*-sabinene hydrate and terpinen-4-ol – decreased significantly after oven drying, while no significant reduction was observed after freeze-drying, suggesting that exposure to elevated temperatures during oven-drying may have influenced the stability of these compounds.

Overall, the results indicate that drying processes can significantly affect the chemical composition of essential oils. Essential oil constituents may undergo chemical transformations such as oxidation, isomerization, cyclization, or dehydration reactions, which can be initiated enzymatically or chemically, leading to changes in molecular structure. Factors

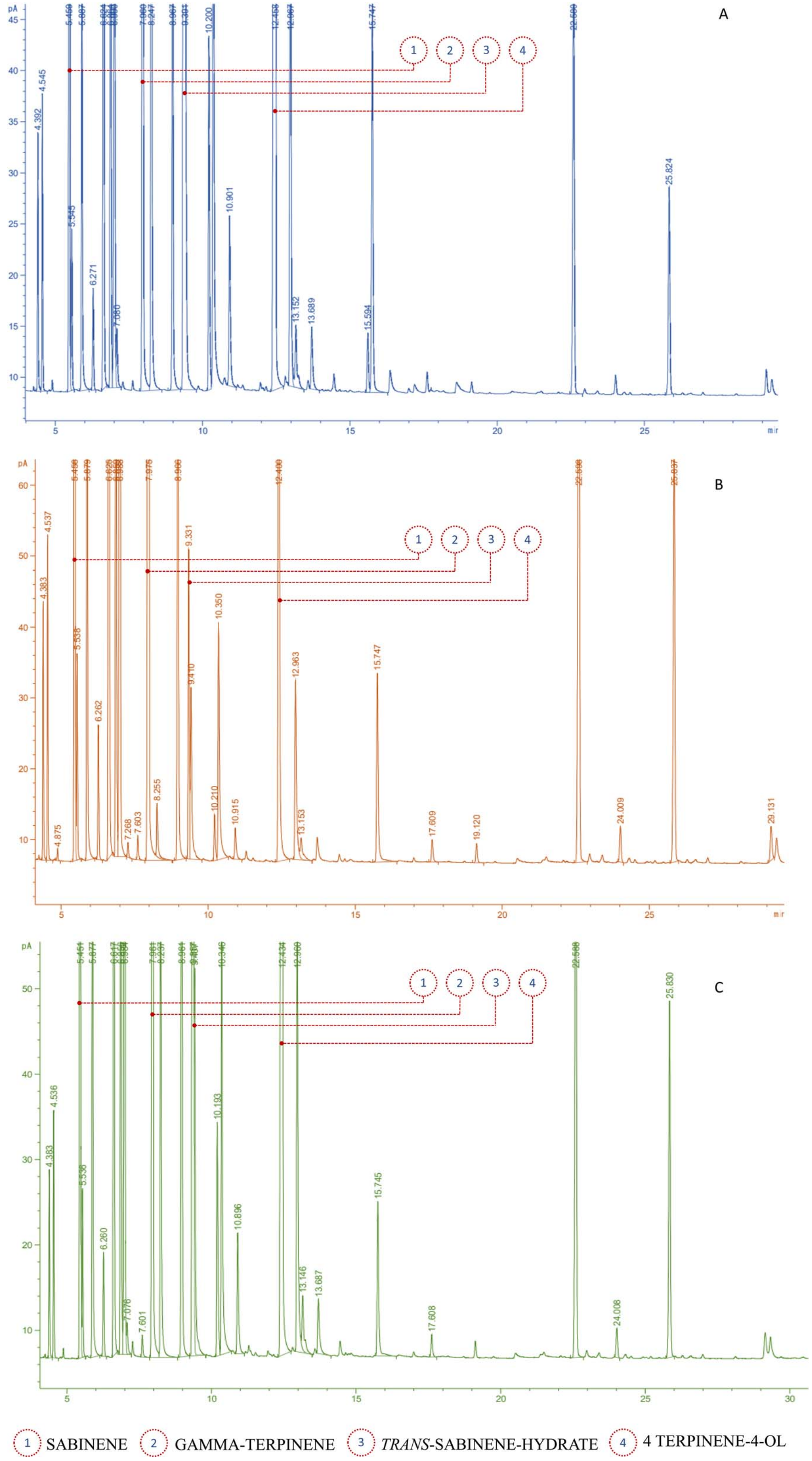

Fig. 1 GC-FID chromatograms of raw marjoram essential oil (A) and encapsulated MEO after drying treatment (oven-drying, (B); freeze-drying, (C)).

such as temperature, light exposure, and oxygen availability may promote oxidative damage, chemical rearrangements, or polymerization reactions, altering essential oil composition.[22,26] In this sense, the application of heat during oven-drying may have favored structural modifications in certain MEO constituents.

Table 1 Chemical composition of raw marjoram essential oil (MEO) and encapsulated MEO after drying (oven-drying, OD; freeze-drying, FD) (mean values ± SD)[a]

Peak	Identification	Class	LRI_{exp}	IRL_{lit}	MEO content (mg g^{-1})	OD content (mg g^{-1})	FD content (mg g^{-1})
1	Alpha-thujene	MT	923	924	8.01 ± 0.17^{b}	14.57 ± 1.95^{a}	10.25 ± 1.38^{b}
2	Alpha-pinene	MT	930	932	10.08 ± 0.20^{b}	18.23 ± 2.45^{a}	12.90 ± 1.73^{b}
3	Camphene	MT	944	946	ND	0.75 ± 0.10^{a}	0.56 ± 0.08^{a}
4	Sabinene*	MT	969	969	102.09 ± 2.33^{b}	196.60 ± 26.38^{a}	152.47 ± 20.46^{a}
5	Beta-pinene	MT	973	974	6.79 ± 0.26^{b}	12.06 ± 1.62^{a}	8.92 ± 1.20^{b}
6	Myrcene	MT	988	988	22.59 ± 0.37^{c}	52.75 ± 7.08^{a}	39.30 ± 5.27^{b}
7	Alpha-phellandrene	MT	1003	1002	4.75 ± 0.18^{c}	9.96 ± 1.34^{a}	7.51 ± 1.01^{b}
8	Alpha-terpinene	MT	1014	1014	87.03 ± 2.00^{b}	185.66 ± 24.91^{a}	141.09 ± 18.93^{a}
9	*p*-Cymene*	MT	1021	1024	16.77 ± 0.32^{b}	56.35 ± 7.56^{a}	44.89 ± 6.02^{a}
10	Limonene* + beta-phellandrene*	MT	1025	1025	47.10 ± 1.11^{c}	108.99 ± 14.62^{a}	81.95 ± 10.99^{b}
11	1.8-Cineole	MO	1027	1026	3.12 ± 0.13^{a}	ND	2.00 ± 0.27^{b}
12	*cis*-Beta-ocimene	MT	1033	1032	ND	1.38 ± 0.19^{a}	0.97 ± 0.13^{b}
13	*trans*-Beta-ocimene	MT	1043	1044	ND	2.30 ± 0.31^{a}	1.74 ± 0.23^{a}
14	Gamma-terpinene	MT	1054	1054	129.17 ± 3.02^{b}	340.08 ± 45.63^{a}	25.51 ± 3.42^{c}
15	*cis*-Sabinene-hydrate*	MO	1062	1065	57.62 ± 1.35^{a}	8.01 ± 1.07^{b}	44.10 ± 5.92^{a}
16	Terpinolene	MO	1085	1088	32.88 ± 0.71^{c}	80.90 ± 10.85^{a}	61.00 ± 8.18^{b}
17	*trans*-Sabinene-hydrate*	MO	1097	1098	295.47 ± 6.47^{a}	32.95 ± 4.42^{b}	212.06 ± 28.45^{a}
18	Linalool	MO	1098	1095	ND	23.10 ± 3.10	ND
19	*p*-*cis*-Ment-2-en-1-ol	MO	1118	1118	19.96 ± 0.35^{a}	5.47 ± 0.73^{a}	18.84 ± 2.53^{a}
20	*p*-*trans*-Ment-2-en-1-ol	MO	1136	1136	11.50 ± 0.20	ND	ND
21	Terpinen-4-ol*	MO	1174	1174	286.29 ± 6.42^{a}	104.37 ± 14.00^{b}	258.46 ± 34.68^{a}
22	Alpha-terpineol	MO	1187	1186	41.33 ± 0.78^{a}	25.76 ± 3.46^{b}	43.80 ± 5.88^{a}
23	*cis*-Piperitol	MO	1192	1195	6.58 ± 0.14^{a}	3.96 ± 0.53^{b}	4.24 ± 0.57^{b}
24	*trans*-Piperitol	MO	1204	1207	4.20 ± 0.03^{a}	3.88 ± 0.52^{a}	6.79 ± 0.91^{a}
25	*trans*-Sabinene hydrate acetate	MO	1250	1253	4.17 ± 0.03	ND	ND
26	Linalyl acetate	MO	1253	1254	28.91 ± 0.59^{a}	21.8 ± 2.93^{b}	21.71 ± 2.91^{b}
27	Terpinen-4-ol acetate	MO	1296	1299	ND	3.33 ± 0.45^{a}	2.84 ± 0.38^{a}
28	*trans*-Caryophyllene	ST	1412	1417	29.03 ± 0.62^{c}	89.74 ± 12.04^{a}	62.85 ± 8.43^{b}
29	Bicyclogermacrene	ST	1487	1494	15.76 ± 0.31^{c}	47.69 ± 6.40^{a}	34.44 ± 4.62^{b}

[a] Compounds listed in order of elution from an HP-5MS capillary column. Those marked (*) also identified by injection of authentic standards. ND – not-detected; LRI_{exp} – experimental linear retention indices; LRI_{lit} – linear retention indices from the literature and the program NIST MS search 2.0; MT – monoterpenoid; MO – oxygenated monoterpenoid; ST – sesquiterpene. Distinct subscript letters in the same row indicate a significant difference (one-way ANOVA or Kruskal–Wallis, $p < 0.05$).

Multiple degradation pathways may occur simultaneously. For instance, Mahanta *et al.* (2021)[27] described four different degradation routes for linalyl acetate, including hydrolysis to linalool; rearrangements *via* a linalyl carbocation yielding monoterpene hydrocarbons such as myrcene and β-ocimene; and cyclization reactions forming compounds such as α-terpineol, terpinen-4-ol, 1,8-cineole, 1,4-cineole, limonene, terpinolene, and α-terpinene; among other pathways. Additionally, γ-terpinene may be converted into *p*-cymene; sabinene hydrate into α-terpineol, terpinen-4-ol, α-terpinene, and/or γ-terpinene; sabinene into β-phellandrene; and linalyl acetate into linalool, α-terpineol, terpinyl acetate, neryl acetate, geranyl acetate, nerol, geraniol, β-myrcene, *trans*-β-ocimene, and/or *cis*-β-ocimene.

Interactions between essential oil constituents and the encapsulating matrix may further influence these transformations. Compounds from essential oils have been reported to form extensive hydrogen-bonding networks with polysaccharide chains, where they are incorporated, particularly through interactions with –OH and –COOH groups present in alginate matrices.[28] Moreover, compounds belonging to different chemical classes may respond distinctly to processing conditions. A comprehensive understanding of compound behavior under specific treatments requires further investigation, including tracking molecular changes using appropriate chemical markers.[29]

To the best of our knowledge, this study represents the first systematic evaluation of the effects of oven-drying and freeze-drying on the chemical composition of marjoram essential oil encapsulated by ionic gelation. Variations in the concentration of essential oil constituents can directly affect aroma, bioactivity, and overall quality, reinforcing the importance of understanding the impact of processing conditions on essential oil composition.[22,26]

3.2 Encapsulation efficiency

The encapsulation efficiency (EE) results indicated that the wet beads retained 60.0% of the essential oil. This result is consistent with the findings of Hosseini *et al.*,[16] who reported encapsulation efficiency values ranging from 52 to 66% for *Satureja hortensis* essential oil encapsulated in alginate beads produced by ionic gelation. Other studies have also investigated the encapsulation of essential oils in beads obtained by ionic gelation, reporting different EE values depending on the wall materials employed. Volić *et al.* (2018)[30] achieved encapsulation efficiencies of 72–80% for thyme oil in alginate-soy protein beads, whereas Dima *et al.* (2013)[31] reported higher efficiencies (92–97%) for coriander essential oil encapsulated in alginate and alginate/chitosan microspheres.

After drying, the freeze-dried (FD) beads exhibited only a slight decrease in EE, retaining the highest amount of essential oil (51.2 ± 1.8%). In contrast, the oven-dried (OD) beads retained only 33.7 ± 1.8% of the essential oil. Similar trends were reported by Chan (2011),[32] who observed higher encapsulation efficiency and oil retention in freeze-dried beads compared to oven-dried samples. The loss of essential oil in the FD samples can be attributed to the partial co-removal of volatile compounds during water sublimation in the freeze-drying process, as described by Gallo *et al.* (2020).[33] Nevertheless, the low temperatures employed during freeze-drying limit essential oil evaporation, resulting in higher EE values compared to OD beads.

In contrast, the greater essential oil loss observed in OD beads can be attributed to the moderate drying temperature (60 °C), which may accelerate essential oil evaporation and diffusion from the polymeric matrix, leading to lower EE. Additionally, oven-drying may induce partial collapse or structural damage to alginate beads, such as crack formation, thereby reducing the protective capacity of the matrix and facilitating oil loss.[34] These structural effects are further discussed in Section 3.3.

Overall, these results demonstrate that the choice of drying method has a significant impact on the encapsulation efficiency of essential oils in alginate beads (Table 2). From the standpoint of encapsulation efficiency alone, freeze-drying appears to be more suitable for preserving the functionality and stability of microencapsulated systems, particularly for thermally sensitive compounds such as essential oils. However, additional parameters such as morphology, stability, and residual moisture must also be considered to ensure the production of beads with properties tailored to their intended applications.

3.3 Morphology

The images obtained from freeze-dried and oven-dried beads containing marjoram essential oil, analyzed by scanning electron microscopy (SEM), are presented in Fig. 2.

Scanning electron microscopy enabled a detailed examination of the surface and internal morphology of the beads, providing high-resolution images and valuable insights into their structural characteristics. This analysis revealed that both drying treatments induced some degree of surface roughness on the beads (Fig. 2).

The oven-dried (OD) beads exhibited a denser and more compact morphology with the internal structure dispersed throughout the bead interior and characterized by fewer and smaller pores (Fig. 2(c–f)). In contrast, freeze-dried (FD) beads displayed a more porous and aerated internal structure, with thinner walls and larger voids (Fig. 2(h–l)). These morphological differences suggest that OD beads may be mechanically more rigid, while FD beads appear more fragile due to their highly porous architecture. Although no mechanical tests were conducted, qualitative observations from SEM images, together with the physical behavior of the beads during manual cutting prior to morphological analysis, support this interpretation. Future studies incorporating texture analysis or compression testing would allow quantitative assessment of these mechanical properties.

Some OD beads exhibited flattened surface regions, associated with contact points where the beads rested on the drying surface during oven drying. Such deformation could potentially be minimized by employing drying techniques that allow particle suspension in the drying medium, such as fluidized-bed drying. FD beads maintained a more uniform external morphology, with softer structures, larger internal pores, and thinner walls.

The inner characteristics of both the OD and FD beads showed a porous structure with the presence of microchannels inside them. In addition to the temperature effects on the essential oil content, the cracks observed on the OD bead surface could have created a pathway that allowed the diffusion of small molecules to the outside medium, leading to EO loss and consequently lower EE, as indicated in the EE analysis.[8,16,35] Although EO release is desirable for some applications, this release should be controlled; otherwise, it could provide unexpected/undesirable effects.

Both OD and FD beads exhibited porous internal structures with the presence of microchannels. In addition to temperature-induced effects on essential oil stability, the surface cracks observed in OD beads may have facilitated the diffusion of small molecules from the bead interior to the external environment, contributing to essential oil losses and the lower encapsulation efficiency observed for these samples.[8,16,35] While controlled release of essential oils can be desirable for certain applications, uncontrolled release may lead to undesirable effects depending on the intended use.

Table 2 Encapsulation efficiency, moisture content, thermal stability, size and circularity of wet, oven-dried, and freeze-dried alginate beads[a]

Beads	EE^{b}	Moisture content^{c} (g/100 g)	T_{onset}^{c}	Circularity^{b}	Area^{b} (mm^{2})	Diameter^{b} (mm)
Wet	60.0 ± 0.0^{a}	95.46 ± 0.11	—	0.888 ± 0.024^{a}	3.071 ± 0.150^{a}	2.099 ± 0.071^{a}
OD	33.7 ± 1.8^{c}	1.56 ± 0.01^{b}	132.01 ± 0.03^{b}	0.869 ± 0.033^{b}	0.764 ± 0.087^{c}	1.110 ± 0.078^{c}
FD	51.2 ± 1.8^{b}	3.98 ± 0.28^{a}	225.20 ± 0.03^{a}	0.861 ± 0.027^{b}	1.444 ± 0.203^{b}	1.518 ± 0.108^{b}

[a] EE – encapsulation efficiency, T_{onset} – temperature that indicates the onset of degradation, OD – oven-dried, FD – freeze-dried. Distinct lowercase letters in each column represent a significant difference between the samples ($p < 0.05$). [b] ANOVA. [c] *T*-test.

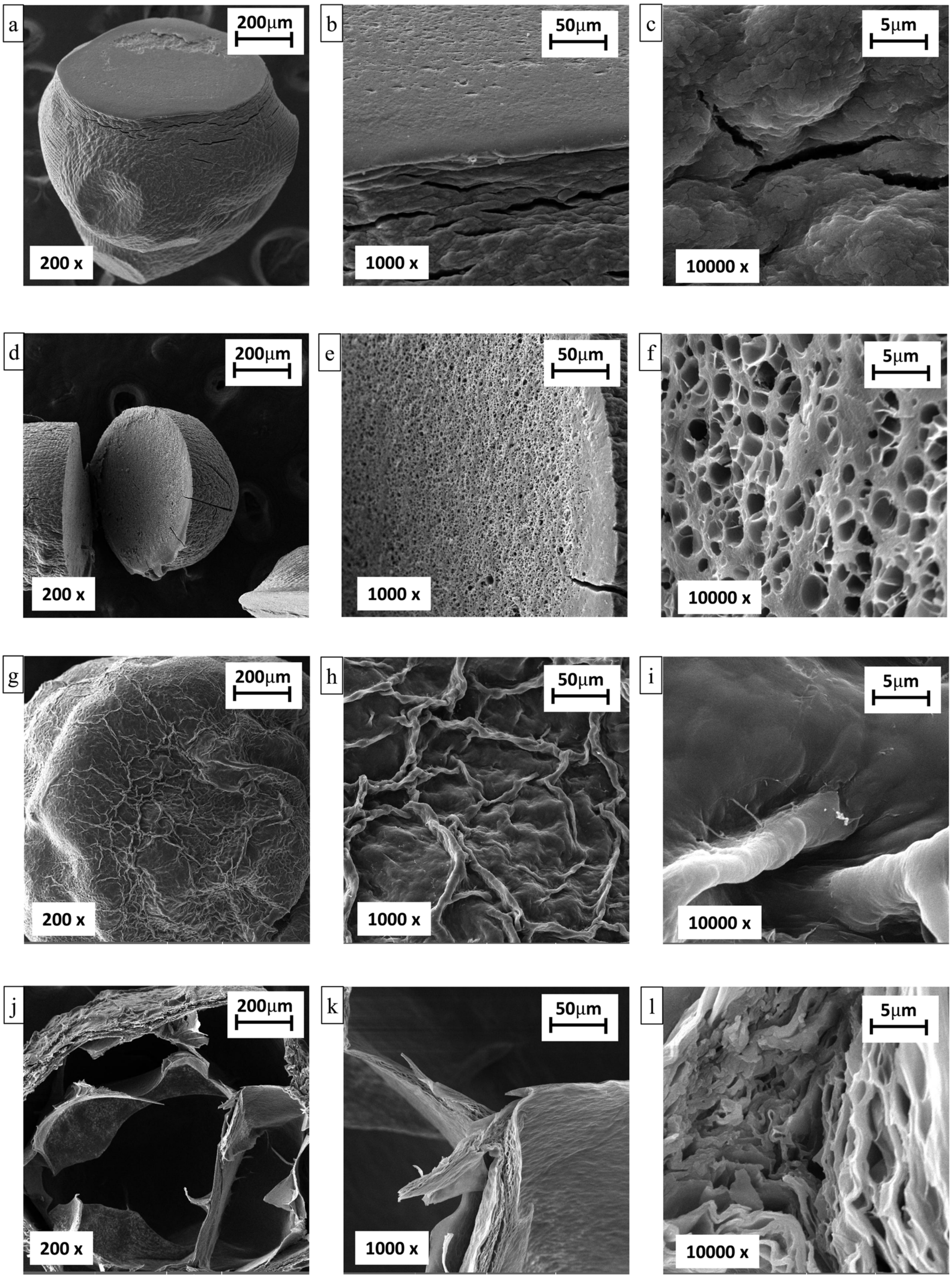

Fig. 2 Structure of the alginate beads after drying treatments obtained by scanning electron microscopy: (a–c) oven-dried (OD) outer structure; (d–f) oven-dried (OD) inner structure; (g–i) freeze-dried (FD) outer structure; (j–l) freeze-dried (FD) inner structure. Magnifications: 200× (bar: 200 μm), 1000× (bar: 10 μm), and 100 00× (bar: 5 μm).

Despite the presence of large internal voids (Fig. 2(j) and (k)), the three-dimensional internal architecture of FD beads allowed improved essential oil retention, possibly due to the relative integrity of the bead surface, which limited diffusion pathways. Kondaveeti *et al.* (2022)[36] also reported that freeze-dried alginate/xanthan blend beads exhibited higher porosity and enhanced adsorption capacity for methylene blue dye compared to air-dried beads.

It is also noteworthy that the oven-dried beads appeared mechanically harder, whereas the freeze-dried beads were more fragile, likely due to differences in internal porosity. These contrasting characteristics may be advantageous or detrimental depending on the target application. Santagapita *et al.* (2011)[37] reported comparable effects of different drying methods (vacuum drying, air drying, and freeze-drying) on the structural properties of alginate beads containing invertase.

Overall, the drying process markedly influences bead morphology, surface features, and internal structure, which in turn may affect functional performance in food, pharmaceutical, and cosmetic applications.[38]

3.4 Size and circularity

Alginate beads typically assume a spherical shape, with dimensions strongly influenced by the preparation method and formulation, as previously reported by Chan *et al.* (2006).[39]

Consequently, variations in processing conditions may affect bead geometry, with potential implications for storage, handling, and end-use applications.

Circularity values (Table 2) provide an indication of how closely the beads resemble a perfect circle, with values approaching 1 representing higher circularity.[40] Results showed that wet beads exhibited higher circularity than both oven-dried and freeze-dried beads. As discussed in the morphological analysis, the drying process altered the external characteristics of the beads, leading to flattened regions in some OD beads and irregular surface features in the FD beads, thereby influencing the circularity.

Both area and diameter can be used to characterize the bead size. Although Feret's diameter is commonly used to estimate particle size, the projected area is particularly suitable for characterizing irregularly shaped beads, as noted by Mazza *et al.*[14] The area parameter represents the two-dimensional space occupied by the beads. Wet beads exhibited the largest mean area, followed by freeze-dried beads, whereas oven-dried beads showed the smallest values. These differences highlight the influence of drying treatments on bead size and morphology and suggest that such effects should be carefully considered when selecting beads for specific applications.

3.5 Moisture content

The assessment of moisture content is crucial for defining appropriate processing and storage conditions for alginate beads, as it directly influences their physicochemical and biological stability.

The initial moisture content of the wet beads was 95.46 ± 0.11 g/100 g. After drying, moisture levels were significantly reduced to 3.98 ± 0.28 g/100 g for the freeze-dried ones and 1.56 ± 0.02 g/100 g for the oven-dried ones, suggesting that this method was more efficient in removing moisture from the beads. This result may be attributed to the incomplete removal of bound water during freeze-drying. In hydrogel-based systems, water molecules can remain tightly associated with the polymer matrix, and sublimation may not have fully eliminated this fraction. The higher moisture content in FD beads may also be attributed to their larger porous structure, as shown in Fig. 2. Other works also reported that the porous structure of the beads affects their moisture content.[32,41] Furthermore, very porous beads can absorb higher amounts of moisture from the environment.

The initial moisture content of the wet beads was 95.46 ± 0.11 g/100 g. After drying, moisture levels were significantly reduced to 3.98 ± 0.28 g/100 g for freeze-dried (FD) beads and to 1.56 ± 0.02 g/100 g for oven-dried (OD) beads, indicating that oven-drying was more effective in removing water under the conditions applied.

Overall, the data obtained in the present study indicated that the moisture content of microencapsulated beads can be effectively controlled through proper drying techniques. High moisture content in the beads had been described to increase the risk of chemical alterations of essential oils; however, this statement was not supported by the literature, as many factors could affect this matter.[26] Nevertheless, the water contained within the beads may exhibit affinity with certain compounds, potentially leading to reduced encapsulation efficiency through diffusion during water removal processes such as freeze-drying (FD) or oven-drying (OD), consequently impacting the quality of the final product.[32]

3.6 Thermal stability

The thermogravimetric analysis (TGA) curves for freeze-dried and oven-dried beads containing marjoram essential oil are depicted in Fig. 3, with the corresponding results summarized in Table 2. The data clearly indicate distinct thermal degradation behaviors between the two drying methods, underscoring the impact of the drying process on the stability of the encapsulated essential oil within the alginate matrix.

The thermal degradation behavior of alginate beads was significantly influenced by the drying method ($p < 0.05$). Oven-dried beads exhibited an earlier onset of degradation (T_{onset}: 132.01 ± 0.03 °C) compared to freeze-dried samples (T_{onset}: 225.20 ± 0.03 °C), suggesting a lower thermal stability of the polymeric network after convective drying.

From a polymer science perspective, exposure to high temperatures during oven drying may promote relaxation, rearrangement, or partial destabilization of the calcium–alginate crosslinked structure, rather than preserving the native junction zones formed during gelation. Such thermal history effects can weaken the integrity of the network, facilitating earlier thermal decomposition.

Previous studies have proposed that heating may induce reorganization of alginate junction zones originally structured as egg-box dimers, potentially leading to altered microstructural arrangements.[42] However, these changes do not necessarily result in enhanced thermal resistance and may instead contribute to heterogeneous or less stable polymer domains. In the absence of complementary structural techniques such as FTIR or DSC, these interpretations should be regarded as indicative rather than definitive.

In contrast, freeze-drying operates under low-temperature conditions that are more likely to preserve the original gel architecture, limiting chain mobility and crosslink rearrangement. This structural preservation is consistent with the higher T_{onset} values observed for freeze-dried beads and has been

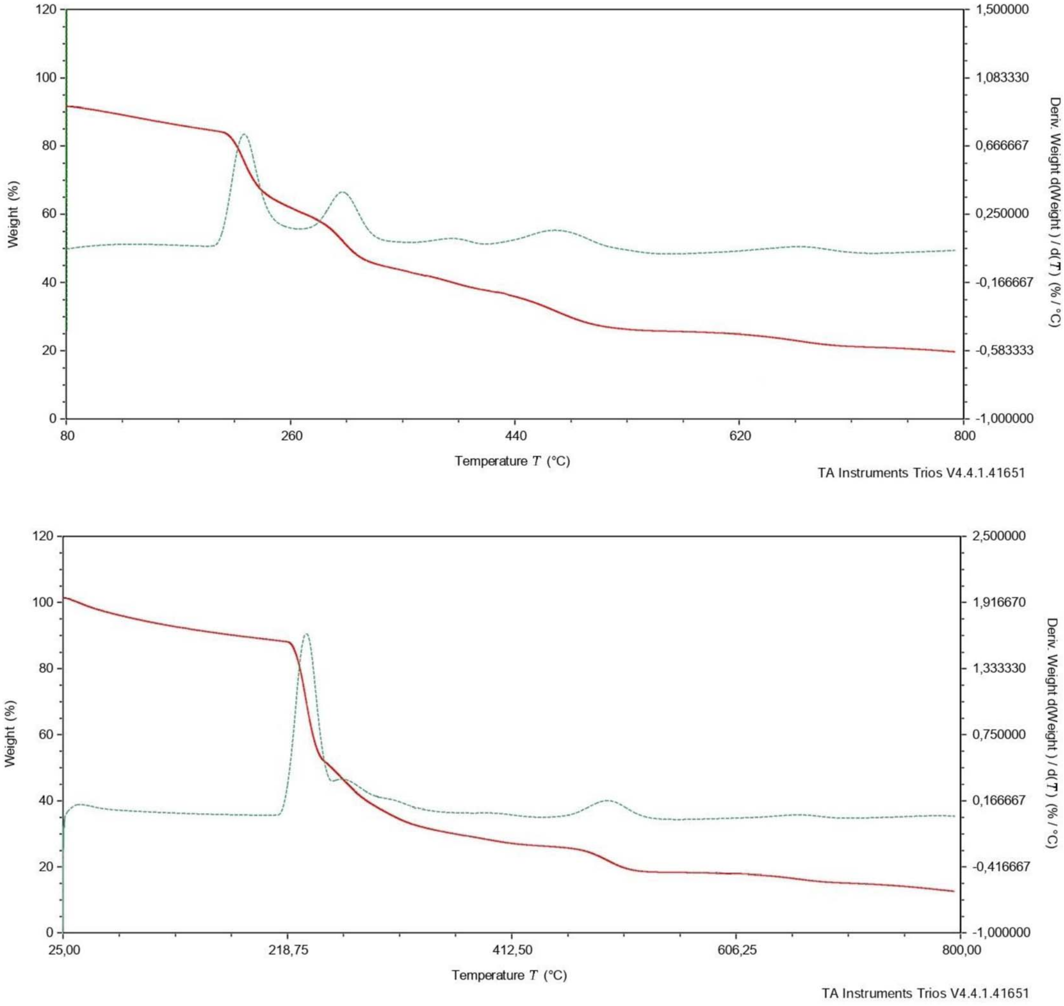

Fig. 3 TGA curves of freeze-dried (FD) and oven-dried (OD) MEO beads.

widely reported for polysaccharide-based hydrogels subjected to different drying routes.

According to Al-Harrasi *et al.* (2022)[43] and Ayouch *et al.* (2020),[38] the first stage of thermal decomposition/weight loss in alginate beads (from approximately 25 to 130 °C) is associated with the evaporation of water. At temperatures above the onset of thermal degradation, microencapsulated beads may begin to degrade,[38,43] leading to a loss of functional properties.

Several studies have investigated the effect of drying techniques and thermal stability in microencapsulated particles. The addition of iron particles during the production of calcium alginate ferrogel beads has been found to reduce thermal stability and promote greater water absorption.[44] Mardaras *et al.* (2021)[45] demonstrated that near-fluidizing microwave drying at lower temperatures enhanced the viability of alginate microcapsules containing living cells. Sampaio *et al.* (2019)[46] studied the encapsulation of a lycopene-rich watermelon concentrate in alginate and pectin beads and evaluated the thermal degradation profile of wet and dried beads through thermogravimetric analysis. The study showed an expected higher thermal stability for the dried beads than the wet ones. It also revealed different mass loss events for the alginate and pectin beads, and their findings suggested both alginate and pectin matrices were effective in preserving lycopene during thermal processing.

These findings highlight the importance of drying methods in determining the thermal properties of alginate-based materials. Although the results suggest that freeze-dried samples may offer advantages in terms of structural preservation and retention of volatile compounds, further studies including release kinetics at elevated temperatures, stability assessments, and application in model food systems, are needed to confirm their suitability for high-temperature applications. These limitations should be considered when interpreting the potential of the encapsulation system.

3.7 Energy consumption estimation

To provide a comparative perspective on process sustainability, the specific energy consumption (SEC) for moisture removal was estimated by dividing the total energy consumed during the drying process (in kWh) by the mass of removed water (in kg). The SEC for freeze-drying was approximately 68.9 kWh kg^{-1} of water removed, based on a benchtop freeze-dryer with combined thermal and electrical power of 2.63 kW operating for 24 hours. Oven-drying at 60 °C consumed around 46.0 kWh kg^{-1}, using a 1.8 kW laboratory oven for the same duration. Therefore, freeze-drying preserved better the essential oil and showed substantially higher energy demand. Nevertheless, these findings should be interpreted with caution, because data were obtained with laboratory scale equipment. The process should reach an industrial scale to provide a more realistic energy consumption estimation.

4 Conclusions

The present study provides valuable insights into the properties of dried beads containing marjoram essential oil, which could have practical applications in the food, pharmaceutical, and cosmetic industries. The drying method was shown to significantly influence the physicochemical and morphological characteristics of the beads.

Freeze-drying was more effective in preserving essential oil content, resulting in beads with higher encapsulation efficiency and greater retention of volatile compounds, but with a more brittle and highly porous structure. In contrast, oven-drying produced denser beads with lower residual moisture and a more compact and organized morphology. These findings indicate that freeze-drying may be more suitable for maintaining the functionality and stability of marjoram essential oil, particularly when the preservation of thermally sensitive constituents is a priority. However, the increased brittleness of freeze-dried beads, along with their higher energy demand, may represent practical limitations. Conversely, oven-drying yields a drier final product, which may be advantageous for applications requiring enhanced moisture control and mechanical robustness.

Future studies investigating sensory attributes, release dynamics, and stability of the encapsulated beads in food systems are essential to determine their practical applicability and to optimize encapsulation and drying techniques based on the desired outcome in food industry applications.

Author contributions

Karen Mazza: conceptualization, investigation, data Curation, formal analysis, visualization, writing – original draft. André Costa: formal analysis, writing – review & editing. Humberto Bizzo: supervision, resources, writing – review and editing. Renata Tonon: supervision, conceptualization, project administration, resources, writing – review and editing, funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Data availability

All data supporting this article are presented within the paper, and all information sourced from the literature has been properly cited.

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