Microencapsulation of spinach extract using binary blends of biopolymers: A comparison between freeze drying and spray drying approaches

Hamid Rajabi (HK.Rajabi@yahoo.com)
Gorgan University of Agricultural Sciences and Natural Resources

Samineh Sedaghati
Gorgan University of Agricultural Sciences and Natural Resources

Ghadir Rajabzadeh
Research Institute of Food Science and Technology

Ali Mohammad Sani
Islamic Azad University Quchan

Research Article

Keywords: Microencapsulation, Arabic gum, Chlorophyll, Spinach

Posted Date: July 17th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3160122/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

This investigation sought to evaluate the relative efficacies of freeze drying (FDM) and spray drying (SDM) methodologies in the microencapsulation of spinach extract, as a source of chlorophylls (CHL), utilizing varying concentrations and ratios of Arabic gum and maltodextrin. Alterations observed in the FTIR spectra substantiated the successful incorporation of CHL within the carriers’ matrix, with the drying method exerting no discernible influence. The mean values for powder yield and encapsulation efficiency in FDM samples at 25% total solid content (TS) were 19.24% and 5.28% greater, respectively, than those of spray-dried samples, while SDM microcapsules exhibited significantly enhanced storage stability. FESEM analysis revealed the considerable impact of drying method, carrier type, concentration, and ratio on both the size and surface properties of samples. An increase in TS from 25–35% resulted in a significant increase in mean particle size for SDM powders from 7.19 µm to 10.96 µm, while FDM samples exhibited an increase in surface roughness. In conclusion, both methodologies demonstrated the capacity to preserve CHL; however, given the significance of energy consumption and process duration at an industrial scale, we propose that SDM represents a suitable approach by producing CHL microparticles with extended shelf-life and favorable processibility.

1- Introduction

Among leafy vegetables, spinach (Spinacea oleracea L.) is of particular importance for human nutrition due to its high content of CHL (a and b), the most prevalent natural pigments (Leite et al., 2018; Li et al., 2021). In recent years, there has been an increasing demand for natural and semi-synthetic derivatives of CHL as food colorants and supplements owing to their functional-salutary properties. CHL have demonstrated antioxidant, anti-mutagenic, and xenobiotic enzyme modulating properties, as well as the ability to induce cancer cell apoptosis in vitro and in vivo (Hayes & Ferruzzi, 2020; Pérez-Gálvez et al., 2020; Zepka et al., 2019; Zhang et al., 2020).

CHL are susceptible compounds that deteriorates when subjected to environmental stressors such as light, heat, and oxygen. In addition, other adverse conditions such as enzymatic reactions or acidic mediums can also impair their quality and their application as functional ingredients is constrained by their poor aqueous solubility (Agarry et al., 2022; Armesto et al., 2017; Hsiao et al., 2020). Therefore, it is imperative to establish a protective method for this beneficial bioactive compound. Encapsulation is a technique that confers protection to sensitive bioactive compounds (core materials) from environmental factors by enveloping them with a layer or incorporating them in a layer called wall materials (Rajabi et al., 2015). Encapsulated herbal products could produce a powder with a prolonged shelf life (Rajabi et al., 2015) and exhibit improved properties in terms of processability, stability, delivery cost, and so forth (Deladino et al., 2008).

Encapsulation is a widely recognized and extensively researched technique that involves creating physical barriers around bioactive compounds to form structures known as “micro/nanocapsules”. The properties of microcapsules, including moisture content, water activity, wettability, solubility, shape and
size, porosity, and their ability to protect the core material (encapsulation efficiency), are determined by the encapsulation method and the properties of the wall material (Rajabi et al., 2015). Furthermore, each encapsulation method produces unique capsules based on factors such as the properties of the wall and core materials, the ratio of core to wall materials, total solid content (TS), drying conditions, and others (Pang et al., 2014). As a result, selecting the appropriate carrier(s) and drying method is crucial in designing a protective system. Spray drying (SDM) and freeze drying (FDM) are two encapsulation methods that employ different mechanisms to protect bioactive compounds. SDM uses higher temperatures for a shorter period of time, similar to an HTST process, while FDM uses much lower temperatures for a longer period of time, similar to an LTLT process. This results in the production of capsules with different properties and levels of protection for the core material. For example, SDM produces nearly spherical particles with smooth or wrinkled surfaces, while FDM produces particles with irregular shapes and surfaces that have a “feathery” or “downy” texture (El-Messery et al., 2020; Šturm et al., 2019). Gum Arabic (GA) and maltodextrin (MD) are common microencapsulation agents that have been used to encapsulate various bioactive compounds such as saffron stigma extract (Rajabi et al., 2015), squalene (Lekshmi et al., 2021), vitamin A (Ribeiro et al., 2020), pumpkin seed oil (Özbek & Ergönül, 2020), juniper berry essential oil (Bajac et al., 2022), and date fruit extract (Arumugham et al., 2023) due to their suitable properties, especially when used in combination.

Due to the unique properties of spinach extract and the factors that limit its application, several studies have investigated its encapsulation using various methods such as spray drying (SDM) (Agarry et al., 2022; Çalıkşan Koç & Nur Dirim, 2017; Kang et al., 2019; Syamila et al., 2019; Zhang et al., 2020), complex coacervation (Agarry et al., 2022), and microfluids (Agarry et al., 2022; Hsiao et al., 2020). To our knowledge, no research has compared the efficiency of SDM and freeze drying (FDM) in improving the stability of CHL. Therefore, we evaluated the behavior of CHL during spray drying/freeze drying encapsulation using binary blends of maltodextrin (MD) and Gum Arabic (GA) as wall materials at two levels of total solid content (TS). The microcapsules were analyzed for water activity, moisture content, powder yield, encapsulation efficiency, and storage stability. Scanning electron microscopy, Fourier transform infrared spectroscopy, and X-ray diffraction were also used to track the changes in the characteristics of the encapsulated powders.

2- Materials and methods

2.1. Spinach extract preparation

The CHL extraction process was conducted in accordance with the methodology delineated by Zhang et al. (2020). Fresh spinach (Spinacia oleracea L.) leaves (500 g), devoid of stems and veins, were rinsed and desiccated. Then, 200 g of the foliage were homogenized in a blender (Pars Khazar, Iran) for 120 seconds with deionized water at a ratio of 1:4 (weight/volume). The resulting puree was subsequently centrifuged at 4000 rpm for 10 minutes at 4°C (Z36HK, Hermle, Germany). The precipitate was then extracted with 400 mL of absolute ethanol and agitated for 30 minutes before being centrifuged at 4000 rpm for 10 minutes to obtain the supernatant containing the CHL. To ensure complete extraction and
recovery of the CHL from the leaves, the extraction procedure was repeated five times until the residue became washed-out. The extracted CHL solution was then concentrated using a rotary evaporator (BUCHI Rotaevaporator R114, Germany) at 40°C for 8 min. The CHL concentration was determined using a UV–Vis spectrophotometer (DR5000, Hach-Lange, Germany) and calculated using the following equations (1 and 2):

\[
\text{Chlorophyll a (µg/mL)} = 11.24A_{662} - 2.04A_{646} \quad (1)
\]

\[
\text{Chlorophyll b (µg/mL)} = 20.13A_{646} - 4.19A_{662} \quad (2)
\]

Wherein \(A_{646}\) represents the absorbance at a wavelength of 646 nm and \(A_{662}\) denotes the absorbance at a wavelength of 662 nm. The total CHL content was ascertained by summing the content of CHL (a and b), and expressed in µg/g dry powder.

GA was obtained from SD Fine Chemical Co. Limited in Mumbai, India. MD, with a dextrose equivalent of 16.5–19, was acquired from Aldrich in the USA. Ethanol was procured from Merck in Germany.

2-2- Preparation of feed composition

The wall materials were apportioned according to the experimental design (Table 1) and incorporated with a stipulated quantity of water at ambient temperature (25 °C) to regulate the final total solids concentration to either 25 or 35% (w/w). The amalgamation was agitated by magnetic stirrer (IKA® C-MAG HS 7) at 400 rpm for 1 h and to ensure complete hydration, feed was refrigerated (5 ± 1°C) for 12 h. Subsequently, the spinach extract was instilled gradually and agitated by stirrer followed by homogenization through rotor-stator homogenizer (Ultra-Turrax IKA® T25) at 14000 rpm for 150 s.
Table 1
Proportions of maltodextrin (MD) and Gum Arabic (GA) according to the mixture design.

<table>
<thead>
<tr>
<th>Wall materials</th>
<th>Amount</th>
<th>MC (%)</th>
<th>aw</th>
<th>PY (%)</th>
<th>EE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FZM</td>
<td>SDM</td>
<td>FZM</td>
<td>SDM</td>
</tr>
<tr>
<td>GA (g) MD (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.25 18.75</td>
<td>25</td>
<td>6.03 ± 1.21</td>
<td>5.05 ± 1.21</td>
<td>0.35 ± 0.01</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>0      25</td>
<td>25</td>
<td>5.06 ± 1.21</td>
<td>4 ± 1.21</td>
<td>0.3 ± 0.01</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>3.125 21.875</td>
<td>25</td>
<td>5.86 ± 1.21</td>
<td>4.8 ± 1.21</td>
<td>0.29 ± 0.01</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>4.6875 20.3125</td>
<td>25</td>
<td>5.98 ± 1.21</td>
<td>4.92 ± 1.21</td>
<td>0.33 ± 0.01</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>4.375 30.625</td>
<td>35</td>
<td>4.9 ± 1.21</td>
<td>3.84 ± 1.21</td>
<td>0.43 ± 0.01</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>6.25 18.75</td>
<td>25</td>
<td>6.07 ± 1.21</td>
<td>5.01 ± 1.21</td>
<td>0.44 ± 0.01</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>4.375 30.625</td>
<td>35</td>
<td>4.92 ± 1.21</td>
<td>3.86 ± 1.21</td>
<td>0.44 ± 0.01</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>8.75 26.25</td>
<td>35</td>
<td>5.25 ± 1.21</td>
<td>4.19 ± 1.21</td>
<td>0.42 ± 0.01</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>1.5625 23.4375</td>
<td>25</td>
<td>5.64 ± 1.21</td>
<td>4.58 ± 1.21</td>
<td>0.3 ± 0.01</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>4.6875 20.3125</td>
<td>25</td>
<td>5.95 ± 1.21</td>
<td>4.89 ± 1.21</td>
<td>0.34 ± 0.01</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>8.75 26.25</td>
<td>35</td>
<td>5.29 ± 1.21</td>
<td>4.23 ± 1.21</td>
<td>0.42 ± 0.01</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>6.5625 28.4375</td>
<td>35</td>
<td>5.03 ± 1.21</td>
<td>3.97 ± 1.21</td>
<td>0.45 ± 0.01</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>2.1875 32.8125</td>
<td>35</td>
<td>4.82 ± 1.21</td>
<td>3.76 ± 1.21</td>
<td>0.38 ± 0.01</td>
<td>0.2 ± 0.01</td>
</tr>
</tbody>
</table>
2-3- Microencapsulation by spray drying (SDM) and freeze drying (FDM)

SDM process was executed through a mini spray dryer (BUCHI, B-191, Switzerland) and the operational conditions for both total solids content were as follow: inlet air temperature 170 ± 5°C, outlet air temperature 85 ± 5°C, air flow 600 l/h, rate of feeding 5 ml/min and atomization pressure 20 psi. The resultant powders were stored to exclude light and oxygen and were conserved at -20 °C until further analysis.

For FDM, the solutions were desiccated utilizing a freeze drier (Operon-Korea) for a duration of 42 hours (−86°C, 5 mbar). The desiccated materials were pulverized with a pestle and mortar and sieved through a 0.71 mm mesh. Subsequently, they were stored in amber glass bottles with screw caps in a freezer (−20 °C) until required for utilization.

2-4- Microencapsulated powder characterization
2-4-1- Moisture content and water activity

The moisture content (MC, % w.b.) of the samples was ascertained by desiccating a measured quantity of powder in a vacuum oven at 70°C until a constant mass was attained and then equilibrating it to the ambient temperature in desiccators with silica gel (Jayasundera et al., 2011). A water activity instrument (LabMaster aw, Novasina, Lachen, Switzerland) was employed to assess the aw of the powders.

2-4-2- Determination of powder yield

To determine the quantity of powder that was squandered during the process, the mass of total soluble content of extract was ratioed to the mass of collected powder from cyclone as given by the following Eq. (3) (León-Martínez et al., 2010):

\[ y = \frac{(W_2 - W_1) - X_{wb} (W_2 - W_1)}{M_T T_s} \times 100 \]  

(3)

Wherein y represents the powder yield (%), Xwb denotes the MC in wet basis (wb), MV signifies the volume of extract feed (L), Ts indicates the content of TS (g dry matter/L), while W1 and W2 represent the weight (g) of the powder holder prior to and subsequent to spray drying, respectively.

2-4-3- Surface CHL and encapsulation efficiency (EE)

The quantity of surface CHL or unencapsulated CHL was ascertained by dissolving 0.05g of powder in 10 ml of hexane and subjecting it to immediate vortexing, succeeded by filtration through a filter paper (Whatman, No. 42). The absorbance of the filtrate was gauged at wavelengths of 662 and 646 nm and the amount of unencapsulated CHL was calculated. For the determination of EE, the CHL content of each sample was ascertained utilizing the methodology delineated by Kang et al. (2019), albeit with slight modifications. A mass of 0.3 grams of each sample was dissolved in 5 ml of distilled water and vortexed for 2 min. Subsequently, 40 ml of an isooctane:isopropanol solution (3:1, v/v) was introduced and thoroughly mixed on a vortex mixer for 1 min. The resulting mixtures were centrifuged (1000 rpm, 10 min), after which the supernatants containing the CHL were rotary evaporated (40°C for 4 min). The concentrated CHL solution was then dissolved in 2 ml of acetone and analyzed spectrophotometrically. Surface and encapsulated CHL were computed utilizing the subsequent equations (4–6) (Zhang et al., 2020):
Whereby the total CHL is the concentration of CHL in the extracted spinach solution before it is added to the feed.

2-4-4- Fourier transform infrared (FTIR) spectra

The chemical groups and bonding arrangements of constituents within the samples were ascertained via Fourier transform infrared spectroscopy (FTIR) utilizing a Perkin-Elmer Spectrum Two (Boston, USA). Measurements were conducted within a wavenumber range of 4000 to 400 cm\(^{-1}\), with 16 scans executed per sample.

2-4-5- X-ray diffraction (XRD) pattern

Phase analysis patterns of samples were elucidated utilizing a X-ray diffraction (XRD, Unisantis, XMD-300, Georgsmarienhütte, Germany) with a Cu K\(\alpha\) radiation (\(\lambda = 0.15418\) nm) over 2\(\theta\) range of 5–40° and scanning rate of 3°/min.

2-4-6- Field Emission Scanning electron microscopy (FESEM)

A field emission scanning electron microscope (FESEM, MIRA3 TESCAN, Brno, Czech Republic) was utilized to assess the morphology of the encapsulated chlorophyll powders. The samples were affixed to specimen stubs with a 2-sided adhesive tape. Subsequently, a thin layer of gold was deposited on the samples under vacuum and the images were acquired at an accelerating voltage of 10.0 kV.

2-4-7- Storage stability evaluation

The proficiency of the encapsulation technique and carriers in safeguarding the CHL against environmental adversities was appraised by preserving the encapsulated powder at temperatures of 4°C and 25°C for a duration of 15 days and quantifying the CHL content at 5-day intervals.

2-5- Statistical analysis
A mixture design employing extreme vertices design with augmented axial points was utilized to obtain diverse ratios of MD and GA for the microencapsulation of CHL extract by SPM and FDM. The experiments encompassed the entire triangular region comprising 26 design points. Data analysis was performed using MINITAB Release 16 (Minitab Inc., PA, USA) software. All experiments were replicated thrice and mean values were reported.

3- Results and discussion

3-1- Effect of wall material ratio, concentration and drying method on microcapsules

3-1-1- Moisture content (MC) and water activity (aw)

The caliber of products hinges on their MC and aw as they impinge on various powder attributes, such as drying efficacy, flowability, adhesiveness, and storage durability. They also modulate the glass transition and crystallization phenomena of the products. The moisture content of the powders varied from 3.96–6.07%, corresponding to the samples produced through SDM-35% MD alone and FDM-6.25GA + 18.75MD, respectively (Table 1). Figure 1 (A) illustrates the variations in MC as influenced by drying methods and TS, both of which exerted a substantial effect on the response. Regarding the role of TS, the MC was markedly diminished in both FDM and SDM samples, a familiar trend that concurred with other research (Mutukuri et al., 2021; Ng et al., 2014; Rajabi et al., 2015). Pertaining to the encapsulation methods, Fig. 1 (A) indicates that SDM produced powders with lower MC than those of FDM. Spray drying generally involves higher temperatures and more water is evaporated during the process, which results in lower moisture content of the spray-dried powder. This effect has been reported by several studies, such as Ishwarya et al. (2022) and Mutukuri et al. (2021). In the same vein, Pellicer et al. (2019) demonstrated that the MC of microencapsulated strawberry flavor varied depending on the drying technique, with spray drying yielding 2.8% and freeze-drying yielding 3.6%.

Having an aw that ranges between 0.2 and 0.4 is recognized for stability against quality deterioration interactions such as browning, lipid oxidation, microbial growth, hydrolytical and enzymatic reactions (Troller, 2012). The aw of the powders ranged from 0.18 to 0.44, respectively for the samples produced via SDM-35% MD exclusively and FDM-6.5GA + 28.5MD (Table 1). Figure 1 (B) illustrates the significant impact of both the encapsulation methods and the TS on the aw. The aw of the SDM samples exhibited a notable reduction as the TS rose from 25 to 35% (w/w), while it reversely affected the FDM particles. Ng et al. (2014) reported that increasing the total solids content of emulsion decreased the aw of encapsulated powder produced by spray drying. This could be due to the higher concentration of solutes in the emulsion, which lowers the vapor pressure of water and thus reduces the aw. Contrary to the anticipated results about the aw of FDM samples, as several authors have documented a decline of aw when augmenting wall materials concentration in powders, this study observed an elevation of aw. Since GA and MD can interact with water molecules through their OH-groups, it was presumed that an
escalation in their concentration could bring about a decrease in aw. A possible explanation for this unexpected trend has been presented by other researchers who attributed the increase of aw to the effect of TS on the emulsion properties, i.e., the higher the TS in emulsion in the frozen state, the more difficult it is for water molecules to spread, giving rise to a decrease in mass-transfer during FDM process (Oberoi & Sogi, 2015). Consistent with these results, Fioramonti et al. (2017) observed that the aw of freeze-dried microencapsulated flaxseed oil increased significantly when the concentration of MD as wall material rose from 10 to 20%. Furthermore, the SDM method generated samples with lower aw than those of FDM method (Fig. 1B). In conformity with these findings, Caliskan and Dirim (2013) and Oberoi et al. (2015), who worked on the encapsulation of sumac extract and watermelon juice respectively, reported that microencapsulated freeze-dried powders had higher aw than those produced through the spray drying approach.

From a stability viewpoint, as shown in Table 1, all the SDM samples had an MC less than 5%, which is considered an appropriate value (Rajabi et al., 2015), and the aw ranged between 0.18–0.27, which could translate to SDM producing stable samples. As for the FDM samples, MC and aw were less than 6% and ranged between 0.29–0.44, respectively, which showed that FDM powders had lower stability than SDM powders, but they still had good stability as their MC and aw were near the optimum values (Fioramonti et al., 2017).

3-1-2- Powder yield

Powder yield is an important factor in microencapsulation as it represents the ratio of the weight of the final dried microcapsules to the weight of the solids (core material and wall material) in the original solution (Calva-Estrada et al., 2018). Powder yield reflects the loss of solids during the drying process. The highest and lowest powder yields correspond to the FDM sample consisting of 8.75GA + 26.25MD (93.5%) and the SDM sample consisting of 6.25GA + 18.75MD (56.95%), respectively (Table 1). A low powder yield means that more of the feed materials are wasted or degraded during drying, which can increase the cost and reduce the quality of the microcapsules (Choudhury et al., 2021). The mean indices of powder yield for CHL are depicted in Fig. 2 (A), and it can be observed that TS exerted a positive influence on the powder yield of both SDM and FDM; that is, higher solids content engendered higher powder yield. Similar results were reported by others (Rajabi et al., 2015; Rezende et al., 2018; Xin et al., 2022). Valková et al. (2022) observed that increasing the total solids content of feed increased the powder yield of spray-dried and freeze-dried β-glucan powder.

On the other hand, the average values of powder yield for FDM powder at both TS contents were significantly higher than those produced via SDM (Fig. 2A). The powder yield of SDM depends on factors that could affect the powder yield, including the feed flow rate during spraying, the temperature at which spray drying is conducted, and the type of material being dried (Rajabi et al., 2015), while for FDM, it depends on factors such as the type and concentration of the core material and carrier, freezing rate, sublimation rate, and drying time (Muhoza et al., 2023). It has been shown that the yield of spray drying
is usually lower than that of freeze drying due to loss of product on the walls of the drying chamber and low capacity of cyclones to separate fine particles (below 2 µm) (Jovanović et al., 2021). Similar results were reported for encapsulation of black glutinous rice (Oryza sativa L.) bran anthocyanins (Laokuldilok & Kanha, 2015), roasted coffee oil Pickering emulsions (Ribeiro et al., 2023), non-dewaxed propolis (Šturm et al., 2019), and strawberry flavor (Pellicer et al., 2019).

3-1-3- Encapsulation efficiency (EE)

Encapsulation efficiency is a measure of how much of the core material was successfully encapsulated and can be used to evaluate the efficiency of the encapsulation process. Encapsulation efficiency values of the microcapsules ranged from 45.9–73.79%, corresponding to the samples produced through SDM-25% MD alone and FDM-8.75GA + 26.25MD, respectively (Table 1). Figure 2 (B) portrays the EE of CHL-included microparticles as affected by TS% and encapsulation methods, in which both TS% and drying methods had a significant influence. Accordingly, increasing the TS% had a positive impact on the retention of CHL by SDM and FDM. It has been demonstrated that the paramount factor governing the preservation of core material during SDM is the feed’s TS. Increasing TSS can increase EE by reducing water content and increasing viscosity of the feed, bringing about a protective barrier to be formed faster around atomized droplets and preventing heat-labile compounds from being destroyed (Rajabi et al., 2015; Tamtürk et al., 2023). In congruence with these data, Mousavi Kalajahi and Ghandiha (2022) worked on spray-drying encapsulation of nettle extract and showed that EE was significantly increased in response to TS increment. In a similar vein, Garofulic et al. (2017) observed that retention of polyphenols from sour cherry juice during spray drying was significantly enhanced by a 3-fold increase in concentration of wall material.

Pertaining to the effect of TS on FDM EE, it affects viscosity, density, freezing point of feed, freezing rate, sublimation rate, and drying time (Ishwarya, 2022; Muhoza et al., 2023). Increasing TS can increase encapsulation efficiency by reducing leakage of core material during freezing and drying (El-Messery et al., 2020). The encapsulation efficiency of bioactive compounds by freeze-drying is contingent on TS, which modulates carrier composition, core-to-carrier ratio, glass transition temperature, viscosity, and droplet size of emulsion. These parameters impact encapsulation capacity, molecular interactions, rigidity and porosity of matrix, which dictate stability, protection and reconstitution of bioactive compounds (El-Messery et al., 2020; Fang & Bhandari, 2012; González-Peña et al., 2021). In conformity with these findings, Azarpazhooh et al. (2018) concluded that increasing concentration of maltodextrin as wall material from 5 to 15% gave rise to a significant increase in encapsulation efficiency of pomegranate peel anthocyanin. More recently, Ruengdech and Siripatrawan (2022) showed that by increasing concentration of wall materials from 5 to 10% (w/w), encapsulation efficiency of catechin significantly increased.

Figure 1 also reveals the effect of the encapsulation method on EE. This Figure illustrates that the EE values of FDM at both TS levels were superior to those of SDM. Regarding the heat sensitivity of CHL on
one hand and the use of high temperature in SDM on the other, it was predictable that FDM had the ability to preserve a higher amount of CHL as it works under very low temperature. These results are consistent with those of previous studies (Dadi et al., 2020; Papoutsis et al., 2018; Ramírez et al., 2015; Rezende et al., 2018; Saikia et al., 2015; Wu et al., 2021). Gue et al. (2020) reported that curcumin encapsulated by freeze-drying presented higher encapsulation efficiency than spray-drying. The paper reported that EE of freeze-drying ranged from 68.8–94.9%, while MEE of spray-drying ranged from 41.1–78.6%. The difference in EE may be due to different operating conditions and wall materials used in each method. In contrast to our result, some researchers claimed that EE of SDM was higher than that of FDM, such as El-Messery et al. (2020) by working on encapsulation of krill oil nanoemulsion, Ballesteros et al. (2017) by working on encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds, and Gomes et al. (2018) by working on encapsulation of papaya pulp. This contrast could be explained by considering the fact that EE by FDM and SDM depends on various factors such as type of compound being encapsulated, wall materials used, and specific conditions of drying process, and shifting from specified conditions from one research to another can bring about different results and trends. For instance, in case of krill oil nanoemulsion, they employed a ternary combination of GA, MD and whey protein concentrate as wall material and applied different drying conditions and core material than the present work, which might have accounted for the discrepancy in results.

3-2- Effect of wall material proportions on the microcapsules

The influence of altering the proportions of wall materials on the characteristics of microencapsulated powders is illustrated in Fig. 3 (A-T). All the responses were significantly affected by varying the amount of MD and GA from the reference blend (87.5%MD + 12.5%GA). Consequently, by augmenting the concentration of MD from the reference blend at both TS levels, and by diminishing GA from that point, MC increased in FDM and SDM microparticles (Fig. 3A-D). Similarly, encapsulation of saffron extract (Rajabi et al., 2015), Sapodilla pulps (Lima et al., 2022), and lemongrass essential oil (Nguyen et al., 2022) using these biopolymers was reported. The water activity of samples exhibited different changes in SDM and FDM at both TS levels. In the FDM powders, aw decreased as the amount of MD and GA respectively augmented and diminished from the reference blend, whereas an inverse effect was observed for SDM microparticles (Fig. 3E-H). The change in aw can be attributed to the chemical structure of GA and MD, which have a high degree of branching with hydrophilic groups and thus, can readily bind the water molecules from the ambient air in the powder (Troller, 2012). Regarding the powder yield, SDM and FDM responded inversely to the alteration in the wall materials proportions, in which the SDM microparticles experienced a decrement trend following the GA proportion augmentation and also MD proportion diminution, while in the case of FDM samples, MD augmentation and GA diminution from the reference blend adversely impacted the powder yield (Fig. 3M-P). Similar results were reported by Rajabi et al. (2015) and Lima et al. (2022) who worked on the microencapsulation of saffron extract and sapodilla pulp, respectively. Increasing GA concentration can enhance the powder yield by forming a more stable and viscous emulsion with the core material as it is capable of forming stable emulsion than MD alone, resulted in higher powder yield. As for EE, GA augmentation from the reference blend at both
TS levels resulted in increasing the EE while augmenting the MD proportions adversely affected it (Fig. 3Q-T). Considering that CHL are hydrophobic compounds and that the GA has a proteinous section, increasing its proportion led to producing an emulsion with higher stability followed by EE augmentation. GA due to its protein fraction can interact with the CHL and form a matrix-type structure that prevents the leakage and loss of CHL (Karaaslan et al., 2021; Todorović et al., 2022).

### 3-3- X-ray analysis

The XRD patterns of MD, GA, CHL, SDM, and FDM powders are shown in Fig. 4. Given that the crystallinity of microcapsules is intrinsically linked to their stability, it is imperative to ascertain whether the microcapsules possess crystalline or amorphous structures via XRD analysis. Typically, the manifestation of broad peaks within the XRD profile is indicative of amorphous structures, owing to the disordered nature of amorphous materials which results in dispersed bands. Conversely, crystalline materials produce sharp and well-defined peaks as a consequence of their highly ordered state.

The XRD profiles of CHL, GA, MD, and CHL-loaded particles encapsulated with GA-MD in varying ratios are depicted in Fig. 4. Two broad and indistinct peaks at $2\theta = 8.6^\circ$ and $19.5^\circ$ were exhibited by CHL, indicative of an amorphous structure with minimal crystallinity. Other researchers reported the same results (Kang et al., 2019). Furthermore, an amorphous structure was also displayed by MD and GA, as evidenced by the presence of broad peaks. Numerous prior studies reported that GA and MD generally possessed amorphous structures (Karrar et al., 2021; Mahdi et al., 2020; Rajabi et al., 2021). In pursuit of evidence to support the objectives of our study, it can be stated that the disappearance of the characteristic peaks of CHL in the XRD profiles of SDM and FDM particles on the one hand and the amorphous structure of CHL-loaded particles on the other provide confirmation that CHL was primarily embedded within the GA-MD matrix. In a similar vein, Liu et al. (2016) reported that the XRD pattern of curcumin underwent a transformation from a crystalline state to an amorphous crystalline state when encapsulated by spray drying. More recently, a study conducted by Zhang et al. (2020) on the encapsulation of CHL through spray drying using different ratios of WPI reported that the characteristic diffraction peaks of CHL almost vanished. This resulted in the formation of a typical amorphous XRD pattern, which clearly indicated the formation of WPI-CHL in the solid-state dispersion.

With regard to the effect of the drying method on the XRD pattern of encapsulated particles, it has been shown that the SDM and FDM processes did not affect the crystalline characteristics of the carriers (Kang et al., 2019; Zhang et al., 2020). These findings are congruent with those obtained from our study. In line with this, Ballesteros et al. (2017) illustrated that the XRD profiles of freeze-dried and spray-dried extracts of spent coffee grounds were similar, with no difference resulting from the encapsulation method.

In relation to the impact of the wall materials ratio on the structure of encapsulated particles, it can be deduced that as the MD content increases (or the GA ratio decreases) in FDM or SDM powders, there is a corresponding progressive increase in the intensity of the XRD peaks. This observation can be attributed
to the fact that the XRD pattern of a matrix more closely resembles that of the ingredient with a higher ratio or concentration (Rajabi et al., 2019). Given that the intensity of the specified peak in MD is greater than that of GA, the peak intensity of microparticles increased in compliance with MD. It is widely recognized that an increased intensity of peaks in an XRD pattern signifies an increased degree of crystallinity of the sample. Consequently, it can be said that powders produced with a higher ratio of MD had more crystallinity. As structural states are associated with shelf life of microcapsules, it can be postulated that storage stability of powders composed solely of MD would surpass that of those produced with a GA/MD blend. Contrarily, storage stability analysis (Fig. 7) exhibited inverse results, potentially attributable to the lipophilic nature of CHL, which necessitates the protein component of GA for formation of a stable emulsion. This factor supersedes crystallinity during storage as it yields higher encapsulation efficiency (higher protection).

### 3-4- FTIR analysis

Infrared spectroscopy was used to determine the changes in functional groups caused by the interaction between MD, GA, and CHL. A comparison of the FTIR spectra for MD, GA, CHL, SDM, and FDM powders is depicted in Fig. 5. The GA spectrum exhibits distinctive peaks at 3457 cm\(^{-1}\) (attributed to O – H stretching vibrations), 2943 cm\(^{-1}\) (C-H stretching), 1619 cm\(^{-1}\) (asymmetric stretching of –COOH and N-H bending), 1425 cm\(^{-1}\) (symmetric stretching of – COOH), and 1291 and 1019 cm\(^{-1}\) (C – O stretching vibrations). The MD spectrum, as illustrated in Fig. 5, exhibited absorption bands at 3419 (corresponding to O-H stretching), 2931 cm\(^{-1}\) (C-H stretching), 1646 cm\(^{-1}\) (asymmetric stretching of – COOH), 1460 cm\(^{-1}\) (CH\(_2\) bending), 1380 cm\(^{-1}\) (O-H bending), 1163, 1078, and 1012 cm\(^{-1}\) (C-O stretching and C-O-H bending), and 940, 839, 756, and 609 cm\(^{-1}\) (skeletal vibrations of the pyranoid ring). These findings are consistent with those reported in prior investigations (Ballesteros et al., 2017; Kang et al., 2019; Rajabi et al., 2021; Rajabi et al., 2020). The distinctive bands of CHL were observed at 3409 (intermolecular bonded alcohol O-H stretching), 2978 cm\(^{-1}\) (intramolecular bonded alcohol O-H stretching), 1719 cm\(^{-1}\) (C-H bending aromatic compounds overtone), 1645 cm\(^{-1}\) (C = N stretching imine/oxime or C = O stretching), 1615 cm\(^{-1}\) (skeletal C = C and C = N stretching of aromatic system in chlorophyll), 1323 cm\(^{-1}\) (O-H bending phenol), and 1069 cm\(^{-1}\) (C-N stretching amine) (Li et al., 2018; Sravan Kumar et al., 2015; Zhang et al., 2020).

A comparative analysis of the FTIR spectra of encapsulated samples fabricated via SDM and FDM techniques revealed that the employed drying methodology exerted negligible influence on the chemical moieties and bonding configurations of the sample constituents. This observation is in agreement with the findings reported by Ballesteros et al. (2017), who investigated the spray/freeze-drying encapsulation of spent coffee grounds extract utilizing MD and GA as encapsulating agents.

The FTIR spectra of FDM and SDM samples exhibit remarkable similarities, yet they differ significantly from those of GA, MD, and CHL in isolation. Upon closer examination of the FTIR spectra of encapsulated powders, it becomes evident that not only has the intensity of certain peaks been altered, but new peaks have also emerged in comparison to MD or GA alone. When juxtaposed with the spectra of MD-GA, a new
absorption peak materializes around 1725 cm\(^{-1}\), corresponding to the C-H bending of aromatic compounds’ overtone originating from the CHL present within the microcapsules. Furthermore, there is a marked increase in the intensity of peaks around 2955 cm\(^{-1}\), potentially attributable to the synergistic effect of O – H stretching in MD, GA, and CHL. Ultimately, the adsorption peaks around 1058 cm\(^{-1}\) become more pronounced and a new adsorption peak emerges at 1145 cm\(^{-1}\), potentially indicative of C-N stretching amine emanating from the CHL. The FTIR spectra of microcapsules encompass the adsorption peaks of both wall materials and CHL, thereby demonstrating that microencapsulation via SD and FD were successfully executed without any discernible chemical interaction between coating and core materials. No chemical interactions were detected between flaxseed oil (Mohseni & Goli, 2019), lavender oil (Ocak, 2012), and coating materials. In a similar vein, Kang et al. (2019) conducted an investigation into the spray drying encapsulation of spinach and discovered that the FTIR spectra of microencapsulated samples constituted a composite of both wall and core materials, thereby corroborating the successful execution of microencapsulation via spray drying. In a more recent development, Karaaslan et al. (2021) undertook a study on the encapsulation of pepper seed oil utilizing GA and MD as wall materials. The findings of this investigation divulged that the fingerprint regions of GA, MD, and pepper seed oil were discernible in the FTIR spectra of microcapsules, thereby signifying the successful implementation of the encapsulation methodology without any chemical bonding transpiring between wall and core materials.

It has been reported that the FTIR peaks could be correlated with the encapsulation efficiency (EE) of microparticles (Karaaslan et al., 2021). The pronounced peaks pertaining to the FTIR spectra of microcapsules epitomized the totality of core material (both surface and encapsulated) within the polymeric structure of wall materials. However, EE results indicated that the proportion of surface CHL relative to total CHL was minimal, suggesting that the observed peaks were more likely attributable to encapsulated CHL rather than surface CHL. Consequently, it can be inferred that the prominent FTIR peaks lend credence to the high EE.

### 3-5- FESEM

The morphological characteristics of SDM and FDM samples are depicted in Fig. 6. The morphology of powders can be evaluated from three distinct perspectives, namely the influence of TS of feed, the ratio between MD and GA, and the encapsulation methodology. These three factors engender alterations in the morphology of microencapsulated powders in terms of size, shape, and porosity. Wall materials type and their concentration are crucial for the production of microparticles by spray drying or freeze drying because they affect the properties of the emulsion (viscosity, surface tension, droplet size and stability of the emulsion) before drying that somehow determine the moisture content, bulk density, and particle size of powders. These factors can affect the drying rate, particle formation and morphology of the microparticles. Moreover, different wall materials may have different interactions with the core material (Fu et al., 2020; Himmetagaoglu et al., 2018; Rajabi et al., 2015; Uekane et al., 2016; Zahran et al., 2022).
It was observed that spinach extract microcapsules produced by SDM exhibited a nearly spherical configuration with varied sizes (Fig. 6A-J), a quintessential attribute of particles obtained via spray drying (Choudhury et al., 2021). Moreover, the particles manifested a relatively uniform morphology and were devoid of any discernible fissures or cracks, indicating efficacious core retention and protection of the encapsulates. Particle size distribution ranged from 2.17 to 22.49 µm and the mean particle diameter for 25% and 35% TS was 7.15 µm and 10.96 µm, respectively. An escalation in TS culminated in larger particle sizes. This phenomenon can be explicated by the viscosity of the feed emulsion, which increased concomitant with solids concentration. This is a typical trend reported by other researchers working on the encapsulation of bioactive compounds (Rajabi et al., 2015). It was shown that the celerity of water extraction from droplets, in conjunction with the type and concentration of wall material and the wall:core ratio, are the predominant determinants of the shape and morphology of spray-dried particles. It is imperative to establish optimal process conditions that facilitate the maximization of the drying rate (Ameri & Maa, 2006).

In light of the impact of wall material ratios on SDM samples, it can be inferred that an escalation in the concentration of GA resulted in an augmenation of surface wrinkling. Consequently, samples synthesized solely with MD exhibited a smoother surface texture. In a similar vein, Akram et al. (2021) conducted research on the encapsulation of fish oil and flaxseed oil and reported that microcapsules produced exclusively with MD possessed a relatively smooth surface in comparison to those produced with GA or a combination of GA-MD, irrespective of the type of encapsulated oil. This phenomenon can be attributed to the higher elasticity of MD in comparison to GA, which can preclude dents or shrinkage induced by mechanical stresses imposed during emulsion drying and water evaporation. Rajabi et al. (2015) demonstrated that the proportion of dented particles in powders produced using MD alone, without the addition of GA, was minimal. However, they observed a linear increase in the percentage of dented particles as the concentration of GA increased.

Upon examination of the FESEM images of FDM and SDM sample, it was observed that the amalgamation of GA and MD as wall materials engendered a transformation in the morphology of the microparticles in comparison to those synthesized solely with MD. The primary distinctions were manifested in the augmentation of the microparticle size when the mixture was utilized, particularly in the case of spray-dried samples. Additionally, the configuration of the microparticles became increasingly irregular and less spherical when the mixture was employed. Moreover, the surface texture of the microparticles also became coarser when the mixture was utilized, irrespective of the technique employed. These observations can be explicated by taking into consideration that the admixture of GA and MD exhibits a higher viscosity than each individual component, which impacts the atomization and drying processes, culminating in larger and less uniform particles. Furthermore, the admixture of GA and MD possesses a lower glass transition temperature than individual MD (Kurozawa et al., 2009), which influences the collapse and shrinkage of particles during drying, resulting in more irregular and coarser particles. Moreover, by escalating the GA ratio in feed concomitant with an increase in protein concentration, not only do molecules and biopolymer chains exhibit a propensity to aggregate and form a compact network through hydrogen bonding, but also the capacity of feed towards water binding is
enhanced. Consequently, in FD, a diminished quantity of ice crystals are formed and in SD, a lesser amount of water is available for evaporation. All these factors contribute to the production of a denser powder with reduced porosity.

For the samples produced using the FDM (Fig. 6K-T), an increase in the TS resulted in a denser and rougher appearance, similar to the effect observed when the concentration of GA was increased. Furthermore, the particle size of FDM powders was significantly larger than those produced using spray drying. This discrepancy in particle size can be attributed to the different powder preparation methods employed. As reported by Xin et al. (2022), the particle sizes of freeze-dried powders were much larger than those of spray-dried powders. This observation is consistent with the findings of Rezende et al. (2018), who suggested that the relatively large size of freeze-dried particles could be due to their low grindability index. In terms of powder porosity, FDM produced samples which their porosity was by far higher than those of spray dried. The porosity of microcapsules plays a crucial role in their functionality within a specific food matrix and is largely dependent on the composition of the microcapsule's wall material and the production technique employed. It has been well demonstrated that FDM produced powders with higher porosity than SDM, as a consequence of the transformation of all water molecules present in the feed into ice during the freezing stage, followed by the sublimation of these crystals during the drying process, a spongy structure is formed (Deshwal et al., 2020; Illa et al., 2019; Muhoza et al., 2023).

3-6- Storage stability evaluation

CHL are lipophilic pigments with high conjugation, which renders them thermally labile during processing and storage. They undergo degradation by various factors such as heat, light, acid, oxygen, and enzymes and easily transform into corresponding pheophytin (Grace et al., 2022). As the objective of encapsulation approaches entails enhancing the stability of bioactive compounds against environmental challenges over time, evaluating the storage stability of microencapsulated powders is essential. The results of assessing CHL stability during 15 days of storage at two temperatures of 4°C and 25°C are presented in Fig. 7. As depicted in this Figure, irrespective of temperature, the stability of CHL in all samples exhibited a downward trend over time, whereby FDM samples demonstrated lower stability than SDM powders. For example, the mean values of SS of CHL in FDM samples at 25% TS and 4°C exhibited a decline from 100% on day 0 to 73.16% on day 15 of storage. In the case of SDM powders subjected to identical conditions, the mean values of SS demonstrated a decrease from 100% on day 0 to 85.86% on day 15 of storage. These results are in line with those reported by Dadi et al. (2020), who worked on encapsulation of Moringa stenopetala leaves extract and concluded that SDM has superior ability over FDM towards protecting bioactive compounds during storage. Another study evaluated storage stability of spray-dried and freeze-dried chitosan-based Pickering emulsions containing roasted coffee oil over 30 days of storage at 25°C. The study found that both drying methods were able to suitably preserve oil quality but spray-drying provided slightly higher protection than freeze-drying (Ribeiro et al., 2023). During SDM, emulsion is propelled through an atomizer and instantaneous drying occurs, enabling MD-GA...
mixture to envelop CHL and form fine particles with smooth or wrinkled surfaces as a firm protective scaffold (Rajabi et al., 2015). On the other hand, FDM creates porous structures through the sublimation of ice crystals. This exposes the structures to oxygen and increases their susceptibility to decay, particularly for lipophilic substances such as CHL (Grace et al., 2022; Özbek & Ergönül, 2020). These outcomes are in accordance with those obtained from morphological analysis of SDM and FDM powders (Fig. 6).

Another factor that influenced storage stability was total solid content. As seen in Fig. 7, a positive influence was observed between TS and CHL stability, in which increasing TS from 25 to 35% significantly improved CHL stability in both SDM and FDM. As shown in the FESEM images (Fig. 6), the particle size of samples was increased following the TS increment from 25–35%. Larger particle size perhaps exposes smaller surface area to the environment (Labuschagne, 2018), so the microcapsules had least exposure to the deteriorating factors gave rise the stability of CHL to be increased over time. Additionally, increasing the total solids (TS) resulted in a denser wall matrix due to increased molecular associations. This limited the penetration of CHL from inside the microcapsule to the surface and oxygen from the surrounding atmosphere into the particles, leading to higher storage stability (Labuschagne, 2018). Moreover, as Fig. 1 (A) illustrates, TS had an inverse relationship with MC. Higher MC meant more molecular movement, which facilitated oxygen penetration and sped up oxidation reactions (Rocha-Parra et al., 2016).

In the case of temperature impact, it adversely affected the CHL stability, regardless of the encapsulation method and TS. As shown in Fig. 7 (H), by increasing the temperature from 4 to 25°C, the storage stability of SDM and FDM powders on the fifth day decreased from 100 to 96.4% and from 99.2 to 91.3%, respectively. The retention of CHL on other storage days showed the same reduction trend (Fig. 7H). The results of the research conducted by Kang et al. (2019) on the storage stability of spray-dried encapsulated CHL using different combinations of GA and MD are consistent with our results. These researchers reported that by increasing the storage time (0–10 days) and temperature (4–40°C), the retention of CHL was significantly reduced. Similarly, Correia et al. (2017) showed that when encapsulated blueberry extract (FDM and SDM) was stored at two temperatures of 4 and 20°C over 16 weeks, both total phenolics and anthocyanins decreased markedly.

The highest stability of CHL at the end of the storage period (95.40%) was observed at 4°C and was achieved by the SDM sample composed of MD:GA at a ratio of 26.25:8.75, while the lowest value (78.5%) was recorded at 25°C for the FDM sample composed only of MD at a concentration of 25% (w/w). To explain these observations, the following points should be considered: 1- CHL are lipophilic pigments and achieving a stable emulsion is one of the determinant factors in increasing their encapsulation efficiency; 2- MD is a modified starch that has no emulsifying ability while GA has a protein fraction that could contribute to emulsion stability; and 3- encapsulation efficiency improved by increasing total solid content. By taking these points into account, it can be said that GA acts as an emulsifier and produces a CHL emulsion that is more stable than those produced with MD alone or those with a lower ratio of GA. This results in the production of microencapsulated powder with higher storage stability.
3-7- possible interactions between wall materials and CHL

According to Phillips and Williams (2009), GA is a complex mixture of glycoproteins and polysaccharides, primarily composed of polymers of arabinose and galactose, with numerous carboxyl and hydroxyl groups on its surface. MD, on the other hand, is a polysaccharide produced by the partial hydrolysis of starch. It consists of D-glucose units connected in chains of varying lengths by $\alpha$-(1→4) and $\alpha$-(1→6) glycosidic bonds (Xiao et al., 2022). CHL is a pigment with a central magnesium atom surrounded by a nitrogen-containing structure known as a porphyrin ring. This ring structure comprises pyrrole rings with nitrogen arranged around a central magnesium ion. Chlorophyll also has a long hydrophobic phytol tail (Prado & Rostagno, 2022). During the encapsulation process, GA and MD can interact with CHL through various mechanisms. For instance, the hydroxyl groups on the polysaccharides can form hydrogen bonds with the polar groups on the CHL molecules. Additionally, hydrophobic interactions can occur between the non-polar regions of the polysaccharides and the hydrophobic regions of the CHL molecules. These interactions can help stabilize the CHL molecules within the microcapsule and protect them from degradation.

The proposed possible interactions between wall materials and core are shown in Fig. 8 (A-C). In the case of MD interaction with GA and CHL (Fig. 8A, C), the hydroxyl groups of the MD molecules can form hydrogen bonds with the carboxyl and hydroxyl groups of the GA molecules, as well as with water molecules. Moreover, hydrogen bonding can occur between the carbonyl groups of maltodextrin and the nitrogen atoms of CHL. Hydrogen bonding can help stabilize the structure and solubility of the microcapsules. Another possible way that these molecules can interact is through hydrophobic interactions. Hydrophobic interactions are a type of non-covalent interaction that occurs when non-polar molecules cluster together in an aqueous environment to minimize their contact with water molecules. Hydrophobic interactions can occur between the phytol tail of CHL and the non-polar regions of maltodextrin. At augmented concentrations ranging from 10–20%, MD gels engender a continuous network via the amalgamation of double helical chains and aggregates of elongated chains (Kanyuck et al., 2019). Furthermore, MD may exhibit an elastic, coiled or helical configuration in aqueous solutions, with the interior of the helical structure being hydrophobic. Owing to its capacity to form complexes with diverse compounds, the structure of MD is susceptible to modulation by coordination complexes (Xiao et al., 2022). Hydrophobic interactions can help protect the CHL from degradation and oxidation by reducing its exposure to water and oxygen. Moreover, the phytol chains of the CHL molecules can interact with each other through van der Waals forces, creating a stable network. While the porphyrin rings of the CHL molecules can interact with the magnesium ions of other CHL molecules through coordination bonds, creating a metalloporphyrin complex (Calogero et al., 2015; Rutkowska-Zbik & Korona, 2012).

In the case of GA interaction with CHL (Fig. 8B), the inclusion of GA into the microcapsule matrix increased the potential for physical interactions between the carriers and CHL. This is due to the possible hydrogen bonds between the hydroxyl and carboxyl groups of GA and the functional groups of CHL, as well as potential interactions between the protein portion of GA and the porphyrin ring of CHL. Previous research has shown that CHL binds to proteinous bodies through non-specific binding by ligation to
amino acids (Agostini et al., 2019; Bednarczyk et al., 2015; Wang et al., 2020). Additionally, GA has a non-polar polypeptide backbone that may interact with the phytol tail of CHL (Fig. 8B). Moreover, the carboxyl and hydroxyl groups of the GA molecules can form hydrogen bonds with water molecules, creating a hydrated layer around the core. These factors contribute to increased protection of the core material against environmental challenges. The reasons behind these observations were forming a dense matrix with the highest possible physical interactions, which was not achieved with MD alone due to its inability to interact with the porphyrin ring. These findings were supported by results obtained from FESEM images, which showed a denser and rougher appearance of microcapsules containing GA compared to those containing only MD, EE analysis, which showed the highest EE at the highest concentration of GA, and storage stability data, which indicated that higher concentrations of GA resulted in greater CHL retention over time.

4- Conclusion

This study assessed the comparative effectiveness of freeze drying and spray drying methodologies in the microencapsulation of spinach extract, employing different concentrations and ratios of GA and MD. The findings revealed that both freeze drying and spray drying techniques exhibited the ability to preserve CHL. An increase in the total solid content and the proportion of GA in the emulsion resulted in an enhancement of encapsulation efficiency and storage stability of the core material. FTIR analysis confirmed the successful incorporation of CHL within the carrier matrix, while FESEM demonstrated the significant influence of wall material type, concentration, and ratio, as well as encapsulation method on the morphological properties of particles. Freeze drying yielded the highest encapsulation efficiency and powder yield, while spray drying produced the highest storage stability and lowest wetting time. Considering the importance of energy consumption and process duration at an industrial scale, spray drying represents an appropriate approach for the production of chlorophyll microparticles with extended shelf-life and favorable processibility as a functional ingredient in food and pharmaceutical industries.

Declarations

Authors’ Contributions Hamid Rajabi: conceptualization, resources, supervision, project administration, funding acquisition, writing—review and editing. Samineh Sedaghati: methodology, validation, formal analysis, writing—original draft. Ghadir Rajabzadeh: Validation, Visualization, Supervision. Ali Mohamadi Sani: Formal analysis.

Funding This work was partially funded by the Ario Rad Mehr Torshiz Company (Mirmohannay).

Data Availability Data will be made available upon request.

Code Availability Not applicable

Compliance with Ethical Standards
Conflict of Interest The authors declare that they have no competing interests

References


the rheological properties, droplet size distribution and microstructure. *Food Research International, 64*, 919-930.


Research, 72, 103115.


Figures
Figure 1
The mean values of MC (A) and aw (B) of microencapsulated powders produced by FDM and SDM at two TS levels of 25% and 35%.

Figure 2
The mean values of powder yield (A) and encapsulation efficiency (B) of microencapsulated powders produced by FDM and SDM at two TS levels of 25% and 35%.
Figure 3

The effect of wall material proportions on the mean values of MC (A-D), aw (E-H), powder yield (I-L), and encapsulation efficiency (M-P) of microencapsulated powders produced by FDM and SDM at two TS levels of 25% and 35%.
The XRD patterns of CHL, GA, MD, and microencapsulated powders produced by FDM and SDM at TS level of 35%.
Figure 5

The FTIR patterns of CHL, GA, MD, and microencapsulated powders produced by FDM and SDM at TS level of 35%.
Figure 6

The FESEM images of microencapsulated powders produced by FDM and SDM at two TS levels of 25% and 35%.
Figure 7

The mean values of CHL retention during storage at 25°C and 4°C and two TS levels of 25% and 35%. (A, B, C, E, F, and G) Comparison between FDM and SDM samples at every 5-day interval and (H) Comparison the rate of CHL loss between FDM and SDM samples during 15 days of storage.

Figure 8

The proposed mechanism of possible interactions between Arabic gum, maltodextrin, and chlorophyll.