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Production of Freeze-Dried Flaxseed Oil Powders by Using Rocket Seed Gum as an Alternative Novel Encapsulation Agent to Improve Oxidative Stability

(Penghasilan Serbuk Minyak Biji Rami Kering Beku menggunakan Gam Biji Roket sebagai Agen Alternatif Enkapsulasi Novel untuk Meningkatkan Kestabilan Oksidatif)

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ABSTRACT

Flaxseed oil rich in α-linolenic acid (ALA) has many health beneficial properties, but suffers from oxidation degradation due to its unsaturated nature, and may need a protective delivery system to apply to different food formulations. In this study, the rocket seed gum was used as a novel encapsulation agent to produce freeze-dried powders. The rocket seed gum (RSG), gum arabic (GA), and their combination were used at two different oil: wall material ratios. Replacing GA with RSG changed the flow behavior of emulsions from Newtonian to shear-thinning, also RSG addition improved the rheological properties of flaxseed emulsion and provided viscoelastic solid characteristics. The encapsulation efficiency (EE %) of flaxseed oil was changed between 38.14 and 52.37%. The effect of wall material type was not significant while the ratio of wall material to oil was significant ($p<0.05$). The FT-IR image of powders showed that flaxseed oil was successfully encapsulated by the RSG. The flaxseed oil powders prepared by RSG showed 3.12 to 5.73 times higher oxidative stability than the flaxseed oil and the powder prepared only with GA. The more amount of air voids observed in SEM images of powders produced with GA might also be related to their lower oxidative stability. Our study showed that rocket seed gum can be successfully used as a new encapsulation agent to produce oxidatively stable microencapsulated flaxseed oil powders.

Keywords: FTIR; microencapsulation; oxidative stability; oxitest; rheology

ABSTRAK

Minyak biji rami yang kaya dengan asid α-linolenik (ALA) mempunyai banyak sifat bermanfaat untuk kesihatan, tetapi mengalami degradasi pengoksidaan kerana sifat tak tepunya dan mungkin memerlukan sistem penghantaran perlindungan untuk digunakan pada formulasi makanan yang berbeza. Dalam kajian ini, gam biji roket digunakan sebagai agen enkapsulasi novel untuk menghasilkan serbuk kering beku. Gam biji roket (RSG), gam arab (GA) dan gabungannya digunakan pada dua minyak berbeza: nisbah bahan dinding. Penggantian GA dengan RSG mengubah tingkah laku aliran emulsi daripada Newtonian kepada penipisan ricih, juga penambahan RSG meningkatkan sifat reologi emulsi biji rami dan memberikan ciri pepejal viskoelastik. Kecekapan enkapsulasi (EE%) minyak biji rami telah diubah antara 38.14% dan 52.37%. Kesan jenis bahan dinding adalah tidak signifikan manakala nisbah bahan dinding kepada minyak adalah signifikan ($p<0.05$). Imej serbuk FT-IR menunjukkan bahawa minyak biji rami berjaya dienkapsulasi oleh RSG. Serbuk minyak biji rami yang disediakan oleh RSG menunjukkan kestabilan oksidatif 3.12 hingga 5.73 kali lebih tinggi daripada minyak biji rami dan serbuk yang disediakan hanya dengan GA. Lebih banyak jumlah lompang udara yang diperhatikan dalam imej SEM serbuk yang dihasilkan dengan GA mungkin juga berkaitan dengan kestabilan oksidatif yang lebih rendah. Kajian kami menunjukkan bahawa gam biji roket boleh berjaya digunakan sebagai agen enkapsulasi baharu untuk menghasilkan serbuk minyak biji rami berkapsul mikro yang stabil secara oksidatif.

Kata kunci: FTIR; kestabilan oksidatif; mikroenkapsulasi; oxitest; reologi

Introduction

Flaxseed oil, with around 60% content of α-linolenic acid (ALA), is considered one of the most important plant omega-3 sources (Kaushik et al. 2016). Although there have been numerous beneficial health effects related to its consumption such as the reduced risk of cardiovascular diseases, hypertension, diabetes, and neurodegenerative disorders, due to its unsaturated nature it is very susceptible to oxidation at led to the loss in quality and nutritional value and subsequently biological functionality (Goyal et al. 2015; Hadad & Goli 2019). To overcome these limitations, a wall barrier between the active molecules and environment to avoid or delay the degradation reactions and therefore improve their stabilization may be created by microencapsulation. Maltodextrin, one of the most common wall materials, offers advantages such as relatively low cost, neutral taste, and odor, providing low viscosity at high solids concentrations, and good protection against oxidation, but suffers from interfacial properties and low emulsifying capacity (Carneiro et al. 2013; Charve & Reineccius 2009). Therefore, for encapsulation purposes, maltodextrin has been partially replaced by other polymers with good emulsifying properties, such as gum arabic (McNamee et al. 2001). However, gum arabic has high price and market sustainability problems (de Barros Fernandes et al. 2014; Krishnan, Kshirsagar & Singhal 2005). The choice of wall material combinations affects both the emulsion properties and the properties of the particles obtained after drying and during storage. It is well known that powder properties such as surface oil, density, morphology, and oxidative stability are affected by the properties of the encapsulating agent used (Carneiro et al. 2013; Ogrodowska, Tańska & Brandt 2017), as well as the emulsion properties such as stability, viscosity, droplet size (Jafari et al. 2008). In recent years, seed endosperm polysaccharides of plants, and galactomannans applied for the use of wall materials to isolate the oil active molecule and limit its mobility (Beikzadeh et al. 2020; Rostamabadi et al. 2022). Galactomannan gums due to their high water-binding capacities can form dispersions with high consistency, and act as effective thickeners and stabilizers (Gadkari et al. 2018). The carbohydrate, protein, moisture, and ash content of RSG were reported to be 80.38%, 5.81%, 10.26%, and 3.55%, respectively (Kutlu et al. 2022). It was also reported that the RSG has a very high galactose substitution level with a 1.52 mannose/galactose ratio. Due to the high protein content of RSG, it has the potential to be used as a natural food hydrocolloid (Hijazi et al. 2022; Koocheki, Razavi & Hesarinejad 2012). Due to the low temperature and vacuum conditions applied during the process, freeze-drying serves as an advantage for the application of many heat-sensitive products such as flaxseed oil (Kouamé et al. 2021). There have been a few studies related to the production of flaxseed oil powders by freeze-drying by using different wall materials such as rice protein concentrate and modified starch (Perrechil et al. 2021), sodium alginate (Zam & Housheh 2019), whey protein isolate, flaxseed musilage and maltodextrin (Fioramonti, Rubiolo & Santiago 2017), and zein (Quispe-Condori, Saldaña & Temelli 2011). Seed gums from natural and renewable sources were reported to have good barrier properties against moisture and oxygen (Beikzadeh et al. 2020). This study aims to investigate the use of rocket seed gum as an alternative agent for the encapsulation of oils. For this purpose, the flaxseed oil emulsions were prepared by using different wall materials, the mixture of maltodextrin, gum arabic, and rocket seed gum, and characterized in terms of droplet size, zeta potential, and rheological properties. Afterward, the emulsions were freeze-dried and the surface morphology, encapsulation efficiency, optical properties, and oxidative stability of flaxseed oil powders were evaluated.

Materials and Methods

Materials

Cold-pressed flaxseed oil was kindly provided by CSK Farma Ltd Sti (Istanbul, Turkey) and stored at 4 °C, maltodextrin (MD, Glucidex IT-19) was provided from the local supplier of Roquettes Frères (Lestrem, France) and gum Arabic powder (GA) was supplied from Vankim (Istanbul, Turkey) and the powders were stored at room conditions. Rocket seeds (Eruca. Sativa Mill) were obtained from Sim Arzuman Seed Products Ltd. Sti (Konya, Turkey) and stored at 4 °C. Rocket seed gum (RSG) was produced according to Koocheki, Razavi and Hesarinejad (2012) with minor modifications. The seeds were ground into powder and extracted with water (1:200; w: v) for 2 h at 80 °C on a magnetic heating mixer (ARE, Velp, USA). Then, the slurry was centrifuged for 10 min at 10,000 g (Centrifuge, Heraeus, Multifuge X3 FR, Thermo Scientific, Germany) to remove the seeds and the supernatant was concentrated by evaporating (Rotavapor, R-100, Buchi, Switzerland) half of the water in the mixture. Afterward, the solution was mixed with two volumes of ethanol to improve the accumulation of the gum on the surface of the solution, and the collected gum was filtered and dried in a drying oven (UN 30, Memmert

GmbH, Germany) at 50 ºC for 24 h to produce Rocket seed gum (RSG). Other chemicals and solvents were were supplied from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA).

MICROENCAPSULATION OF FLAXSEED OIL

All wall materials were dissolved in distilled water for 16 h for complete hydration. The flaxseed oil emulsion preparation conditions of Fioramonti, Rubiolo and Santiago (2017) were modified. The wall materials were mixed with oil at predefined ratios and homogenized at 20.000 rpm for 3 min by using the Ultraturrax homogenizer (Daihan, HG-15, South Korea). Afterward, the coarse emulsions were transferred to a double-walled cylindrical glass beaker and exposed to ultrasonic homogenization (UIP1000hdT, Hielscher, Germany) operating at 20 kHz frequency, 250 W power for 4 min, and the temperature of the emulsion was kept at 15 ºC through the continuous circulation of the cold water around the beaker. The conditions of homogenization were previously determined based on preliminary experiments where different times of sonication were applied (1 - 10 min.) and increasing the homogenization time over 4 min did not further reduce the size of droplets. Emulsions were prepared in 1:2 and 1:4, oil: wall (w:w) ratios with different combinations of RSG. The six different emulsion formulations were presented in Table 1. The emulsions were placed on aluminum dishes and frozen at -80 °C for 24 h, and dried with a freeze drier (Beta 1-8 LSC Plus, Christ) at -50 ºC, 0.1 bar for 48 h. The dried samples were ground to obtain fine powder and stored in brown glass jars with screwed caps at 18 ºC until the analysis.

TABLE 1. The formulations and content of flaxseed oil emulsions used in the study

o:w	Sample codes	Oil (%)	RSG (%)	GA (%)	MD (%)	Water (%)
1:2	F1.2	10	3	-	17	70
	F2.2		2.25	0.75		
	F3.2		0.75	2.25		
	F4.2		-	3		
1:4	F1.4	6	3	-	21	70
	F2.4		2.25	0.75		
	F3.4		0.75	2.25		
	F4.4		-	3		

o:w, flaxseed oil: wall material ratio (w:w); RSG, rocket seed gum; GA, gum arabic; MD, maltodextrin

CHARACTERIZATION OF EMULSIONS

Emulsion Flow Characteristics and Dynamic Rheology Properties

The rheological properties of six emulsion formulations were determined in the range of 0-100 (1/s) shear rate at room temperature using parallel plate configuration (diameter 50 mm, pitch 0.5 mm). Around 2 g of each sample was placed on the rheometer plate and the analysis was carried out after the desired temperature was reached. The shear stress values of the solutions (τ, Pa) were determined as a function of the shear rate (γ, s^{-1}) by using the Power Law model and nonlinear regression.

$$\tau = K\gamma^{n} \quad (1)$$

where τ is the shear stress (Pa); K is the consistency coefficient ($Pa.s^{n}$); γ is the shear rate ($s^{-1.}$); and *n* is the flow behavior index.

Dynamic rheological analyzes of emulsion samples were carried out using the parallel plate configuration.

Firstly, the amplitude sweep test was carried out in the range of 0.1% and 100% strains to determine the linear viscoelastic region. According to this determined value, the frequency sweep test was determined between 0.1-10 Hz frequency and 0.1-64 (ω) angular speed range. Elastic modulus (*G'*), viscose modulus (*G''*), and the complex viscosity (η^*) values were measured against angular velocity and frequency values. Parameters related to dynamic rheological properties were determined by using the Power Law model and non-linear regression (Yoo & Rao 1996)

$$G' = K'(\omega)^{n'} \tag{2}$$

$$G'' = K''(\omega)^{n''} \tag{3}$$

G' value of elastic modulus (Pa), *G''* value viscous module (Pa), η^* value complex viscosity (Pa.s), ω angular velocity value (s^{-1}), K', K'', consistency coefficient values ($Pa.s^n$) and n', n'' values represent the flow behavior index values.

ζ-potential and Particle Size Distribution Measurement

The emulsions were diluted by 1000 times with distilled water, dispersed in an ultrasonic bath (Daihan, WUC-D10H, South Korea) for 1 min, and loaded into the disposable cuvette of a particle electrophoresis instrument (Nano ZS, Malvern Instruments, Malvern, UK) and measurement was done at 25 ºC from three prepared samples, with three readings made per sample. For particle size measurement a refractive index of the material of oil and water was set at 1.4694 and 1.3333, respectively (Tekin et al. 2020).

Optical Microscopy

Light microscopy images of emulsions were taken with a digital camera (DP27, Microscope Digital Camera) mounted on an optical microscope (Olympus, Germany). For this purpose, a few drops of emulsion were dropped on the coverslip and covered with a slide that ensures no air or bubbles and was displayed at 40× objective magnification (Tekin et al. 2020).

CHARACTERIZATION OF FLAXSEED OIL MICROCAPSULES

Moisture Content and Water Activity

The moisture content of flaxseed microcapsules has been determined by a moisture analyzer (MA-50.R, Radwag, Poland). The water activity of the samples was measured by the AW Sprint TH500 Water Activity Meter (Novasina, Switzerland) at 25 ºC (Karadag et al. 2013).

Encapsulation Efficiency (EE%)

Encapsulation efficiency (EE%) was determined according to the method of Fioramonti, Rubiolo and Santiago (2017) and Quispe-Condori, Saldaña and Temelli (2011) with some modifications. The non-encapsulated oil present on the surface of microcapsules was extracted by mixing the powder (5 g) and hexane (50 mL) in a sealed glass container at room temperature for 15 min. Then, it was filtered through Whatman No.1 filter paper and the powder that remained on the filter paper was three times washed with 20 mL of hexane, and the collected solvent was removed under a fume hood for 24 h and placed in an oven at 60 ºC until the constant weight reached. The weight difference between the initial clean flask containing oil residue was determined as the amount of oil on the powder surface. For the determination of total oil content which includes both encapsulated and non-encapsulated oil, the microencapsulated powder (1 g) was dissolved in water (10 mL), stirred gently, and placed in an ultrasonic bath (VWR, Ultrasonic Cleaners, USA) at room temperature for 15 min. The sample was extracted three times with a total of 15 mL of hexane, and each time upper hexane phase was collected in a flask and the solvent was removed, and the total oil content was determined gravimetrically as done before. Encapsulation efficiency (%) was calculated according to equation (4)

$$EE\% = \frac{Total\ oil - Surface\ oil}{Total\ oil} * 100 \tag{4}$$

Colour (L, a*, and b* values)*

The colour values of the microencapsulated flaxseed oil were measured using a chromameter (Konica Minolta CR-400, NJ, USA). They were expressed as L* (whiteness/darkness), a* (redness/greenness), and b* (yellowness/blueness) (Goztepe et al. 2022).

Morphological Analysis

Freeze-dried powders were mounted onto separate, adhesive-coated aluminum pin stubs. The excess powder was removed by tapping the stubs sharply and then blowing dry air across. The stubs were sputter-coated with a thin layer of gold, and the samples were examined using a Scanning Electron Microscope (SEM, EVO LS 10,

Carl Zeiss, Germany) operated at a high vacuum with an accelerating voltage of 7 kV with a working distance of 9 mm. Images were taken at 1000 and 3000 magnifications (Cihat Icyer et al. 2017).

Fourier Transform Infrared Spectroscopy (FTIR)

The chemical characterization of flaxseed oil microcapsules was measured by FTIR (Bruker Tensor 27, Bremen, Germany) spectroscopy equipped with a MIR TGS detector and ATR beam separator. The measurement was recorded as the average of 16 scans at frequencies between 650-4000 cm^{-1} with a resolution of 4 cm^{-1} (Karakas et al. 2022).

Oxidative Stability

The oxidative stability of flaxseed oil and microcapsules was monitored by OXITEST (Velp Scientifica, Usmate, Milan, Italy) (Akcicek et al. 2021) equipped with two separated oxidation chambers. After uniformly dispersing the sample (8 g) in the chamber, it was hermetically sealed, heated to 90 °C and pressurized oxygen (99.9999% purity) was injected into the chamber. The analysis was initiated after the oxygen pressure reached 6 atm. The Oxitest reactor monitors the absolute pressure change inside the chambers calculating the oxygen uptake of the oxidizable compounds of the samples and automatically generates the Induction Period (IP). The higher IP value shows the higher resistance of the sample to oxidation.

STATISTICAL ANALYSIS

All measurements were repeated at least 3 times using duplicate samples, and the results were given as the means and standard deviations. One-way analysis of variance (ANOVA) was conducted using the SAS Institute package computer program (Cary, NC, USA). Differences were analyzed using Duncan's Multiple Range Test comparisons and the p-value of <0.05 was chosen to determine the significant differences. For rheological analysis, the Power-law model parameters were calculated with the help of non-linear regression analysis by the Statistica software program (Stat Soft Inc., USA).

Results and Discussion

CHARACTERIZATION OF EMULSIONS

Rheological Properties of the Emulsions

The rheological characteristic of the emulsions is an important parameter that affects droplet diameters and powder characteristics of the microparticles (Xie et al. 2010). Figure 1 shows the steady shear rheological properties of the emulsions. The slope of the shear rate versus shear stress graph of emulsions prepared with RSG and RSG/GA combination showed a decreasing trend with increasing shear rate. In other words, the viscosity value of the emulsions prepared with RSG and RSG/GA combinations reduced with increased shear rate, indicating the shear-thinning flow behavior of emulsions. The shear-thinning behavior of rocket seed

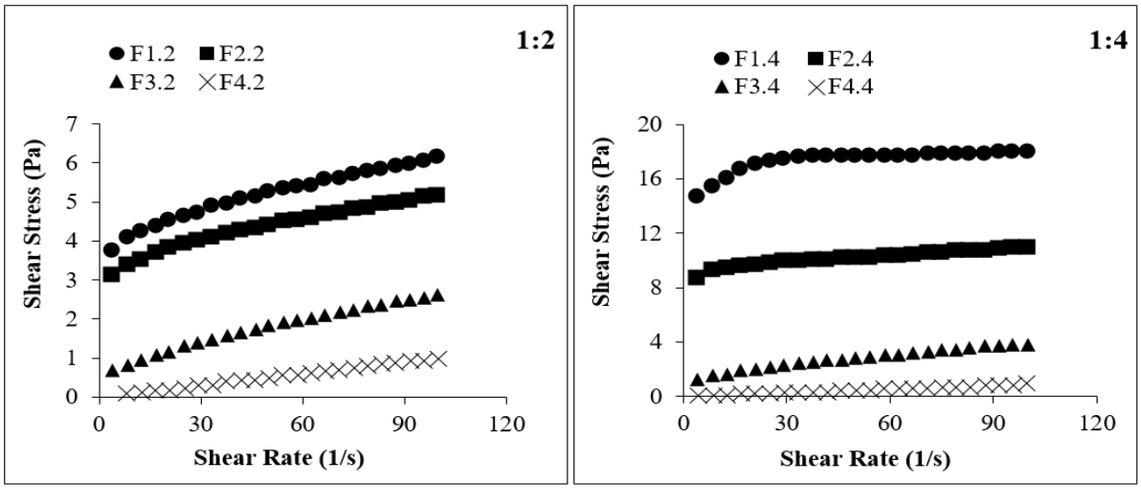

FIGURE 1. Steady shear rheological properties of the emulsion formulations. 1:2 and 1:4 refers the to oil: wall material ratio of 1:2 and 1:4 (w:w). In each o:w ratio, from F1 to 4 RSG concentrations changed from 3 to 0% while GA increased from 0 to 3%

and some natural gums was also reported in previous studies (Akcicek et al. 2022; Bhushette & Annapure 2018; Wang et al. 2019). The shear-thinning character of emulsions could be due to the breakdown of the intermolecular interaction between highly branched polysaccharides, protein, and water as a consequence of induced shear (Quemada & Berli 2002). The slope of the emulsions prepared without RSG, but only with GA, did not change by increasing the shear rate, indicating Newtonian flow behavior of GA emulsions. Very small changes in viscosity and flow behavior indices of GA emulsions were also noted previously (Ramaswamy et al. 2020). The flow behavior characteristic of the emulsions was successfully modeled (R^2>0.92) by the Power Law model. The model parameters, K, and n values were presented in Table 2. By increasing the RSG content in the emulsions, the higher K value accompanied lower n values, indicating that RSG provided more consistency and increased pseudoplastic characters to the emulsions. However, in emulsions with high GA content, regardless of oil: wall material ratio, very low K value (0.01-0.02) and high n value (closed to 1) were observed, indicating that GA emulsions showed very low consistency. In the study of Ahmed, Ramaswamy and Ngadi (2005), the addition of GA reduced the rheological properties of both guar and xanthan gum.

TABLE 2. The Power-Law parameters defining flow behavior properties of emulsions

		$\sigma = K\gamma^n$		
o:w	Sample codes	*K*	*n*	R^2
1:2	F1.2	2.76 ± 0.02^{Ab}	0.17 ± 0.01^{Ca}	0.99 ± 0.00^{Ba}
	F2.2	2.28 ± 0.84^{Bb}	0.17 ± 0.01^{Ca}	0.99 ± 0.00^{ABa}
	F3.2	0.27 ± 0.05^{Cb}	0.49 ± 0.04^{Ba}	0.99 ± 0.00^{ABa}
	F4.2	0.02 ± 0.00^{Da}	0.91 ± 0.05^{Aa}	0.99 ± 0.00^{Aa}
1:4	F1.4	14.06 ± 1.33^{Aa}	0.06 ± 0.02^{Cb}	0.92 ± 0.04^{Ba}
	F2.4	7.89 ± 0.35^{Ba}	0.07 ± 0.01^{Cb}	0.99 ± 0.00^{ABa}
	F3.4	0.62 ± 0.03^{Ca}	0.39 ± 0.00^{Bb}	0.99 ± 0.00^{Aa}
	F4.4	0.01 ± 0.00^{Ca}	0.96 ± 0.04^{Aa}	0.99 ± 0.00^{Aa}

Results were given as mean±SD. Different uppercase letters indicate the difference for the samples with the same oil: wall material ratio (o:w); different lowercase letters indicate the difference for the samples with different oil: wall material ratios ($p<0.05$). In each o:w ratio, from F1 to 4 RSG concentrations changed from 3 to 0% while GA increased from 0 to 3%

Figure 2 shows the dynamic rheological behavior of the emulsions. In all frequency ranges, the G′ value of the RSG and RSG/GA emulsions was higher than the G″ value, therefore it could be said that emulsions prepared with RSG showed viscoelastic solid characters, which elevated by higher RSG content in wall material. The emulsions prepared with the only GA showed a lower G′ value than G″ which indicated the liquid-like behavior of GA emulsions. The Power-law model parameters, K′, K,″ and n′, n″ values were presented in Table 3. The higher value of K′ was obtained by elevating the RSG content of the emulsions, therefore RSG provided strong viscoelastic solid characters. K″ value of the GA emulsions for oil: wall material ratios of 1:2 was significantly higher than K′ ($p<0.05$), confirming the liquid-like behaviour and the weak structure of GA emulsions.

TABLE 3. Power-law model parameters defining dynamic rheological properties of emulsions

		$G' = K'(\omega)^{n'}$			$G'' = K''(\omega)^{n''}$		
o:w	Sample codes	K'	n'	R^2	K''	n''	R^2
1:2	F1.2	2.06 ± 1.74^{Abx}	0.55 ± 0.34^{Ba}	0.99 ± 0.01^{Aa}	0.79 ± 0.02^{Aby}	0.53 ± 0.03^{Ba}	0.982 ± 0.01^{Aa}
	F2.2	1.87 ± 2.32^{ABbx}	0.56 ± 0.72^{Ba}	0.63 ± 0.50^{Bb}	0.40 ± 0.02^{Bby}	0.67 ± 0.04^{Ba}	0.979 ± 0.01^{Aa}
	F3.2	0.10 ± 0.12^{Bax}	1.18 ± 0.55^{Ba}	0.99 ± 0.02^{Aa}	0.15 ± 0.16^{Cax}	0.84 ± 0.35^{Aba}	0.928 ± 0.05^{Ba}
	F4.2	0.00 ± 0.00^{Bay}	2.07 ± 0.16^{Aa}	0.99 ± 0.01^{Aa}	0.02 ± 0.02^{Dax}	1.16 ± 0.40^{Ab}	0.872 ± 0.02^{Ca}
1:4	F1.4	14.66 ± 0.73^{Aax}	0.27 ± 0.01^{Ca}	0.98 ± 0.00^{Ba}	3.26 ± 0.03^{Aay}	0.40 ± 0.00^{Db}	0.98 ± 0.01^{Aa}
	F2.4	5.55 ± 1.00^{Bax}	0.38 ± 0.04^{Ca}	0.98 ± 0.01^{Ba}	1.58 ± 0.04^{Bay}	0.48 ± 0.01^{Cb}	0.98 ± 0.01^{Aa}
	F3.4	0.17 ± 0.07^{Cax}	0.92 ± 0.17^{Ba}	0.98 ± 0.01^{Ba}	0.19 ± 0.00^{Cax}	0.76 ± 0.01^{Ba}	0.98 ± 0.00^{Aa}
	F4.4	0.00 ± 0.00^{Cax}	2.07 ± 0.07^{Aa}	0.99 ± 0.00^{Aa}	0.00 ± 2.22^{Dax}	1.96 ± 0.02^{Aa}	0.88 ± 0.11^{Ba}

Results were given as mean±SD. Different uppercase letters indicate the difference for the samples with the same oil: wall material ratio (o:w); different lowercase letters (a-b) indicate the difference for the samples with different oil: wall material ratios ($p<0.05$); different lowercase letters (x-y) indicate the difference between K' and K'' value for the same samples ($p<0.05$). In each o:w ratio, from F1 to 4 RSG concentrations changed from 3 to 0% while GA increased from 0 to 3%

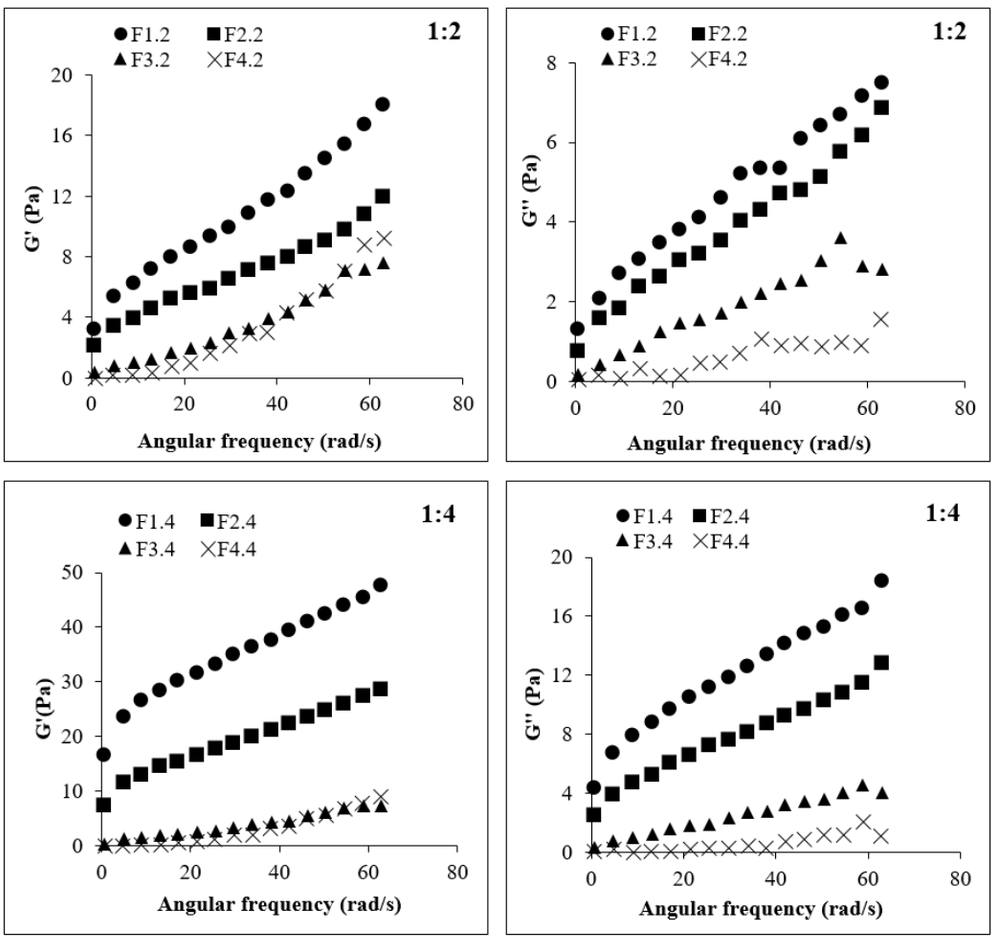

FIGURE 2. Viscoelastic behavior of the emulsion formulations. 1:2 and 1:4 refers to the oil: wall material ratio of 1:2 and 1:4 (w:w). In each o:w ratio, from F1 to 4 RSG concentrations changed from 3 to 0% while GA increased from 0 to 3%

Particle Size and Zeta Potential Value

The particle size and zeta potential values of emulsions were presented in Table 4. Although electrostatic repulsion was less important in terms of providing long-term stability when the emulsions were stabilized by polysaccharides that produce thick interfacial layers that generate long range steric repulsion, the charge of droplets may still be important to expel the water-soluble ions (transition metals) that interacts with the oil droplets, therefore, reducing the possible chemical oxidation reactions (McClements & Jafari 2018; Piorkowski & McClements 2014). In our study, the zeta potential values of all formulations were changed between -25.7 and -31.9 mV, as both RSG and GA presented anionic properties. The emulsions prepared only with GA showed the lowest particle size in high oil: wall material ratio and the droplet size of emulsions increased at higher RSG concentrations. At high wall material and with less oil ratio (1:4; o:w), the concentration of RSG was not significant on the droplet size of RSG/GA emulsions. Whereas in the presence of more oil and low wall material (1:2; o:w), the RSG concentration was significantly important to the droplet size. After a certain concentration of RSG addition (>0.75%, w/w), the droplets became larger ($p<0.05$), so it could be said that when there were more oil-water surfaces to cover, the amount of GA in the formulation may not be enough. In the emulsion formulations when gum Arabic was mixed with other gums, it would display its surface-active properties whereas other gums would mainly be responsible for the thickening of the continuous phase, therefore, restrict the movements of droplets and contributing to the emulsion stability (Desplanques et al. 2012). It has also been reported that when the mixture of two hydrocolloids is used in emulsions, concentration of gums, their molecular weight, functional groups and the degree of interaction between two hydrocolloids are important for the resulting properties of emulsions (Ahmed, Ramaswamy & Ngadi 2005).

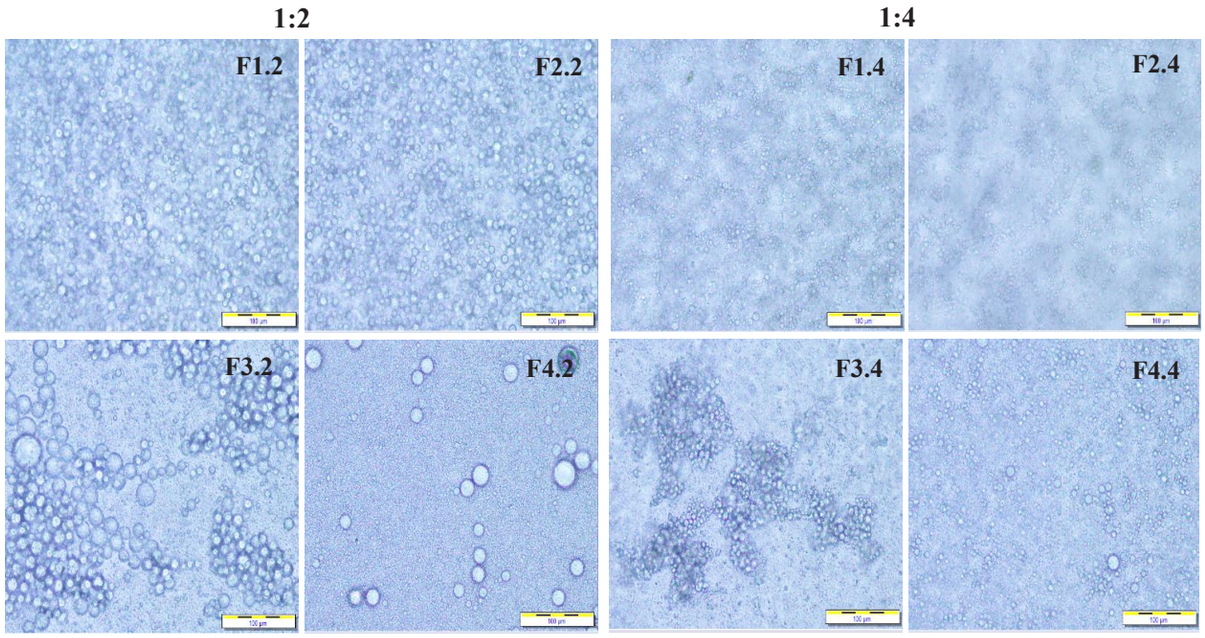

FIGURE 3. Optical microscope images of emulsion formulations. 1:2 and 1:4 refers to the oil: wall material ratio of 1:2 and 1:4 (w:w). In each o:w ratio, from F1 to 4 RSG concentrations changed from 3 to 0% while GA increased from 0 to 3%. The scale bar corresponds to 100 µm

The optical microscope images (Figure 3) showed the presence of some flocs while adding RSG (0.75%) into the formulation, but they were reduced by increasing the amount of RSG which could be related to providing higher viscosity (Figure 1). It was reported that when amphiphilic polysaccharides were used above a certain level, they can show depletion flocculation and at sufficiently high concentrations a three-dimensional network was formed that traps the droplets and effectively prevents their movement and contact with each other (Bai et al. 2017). Light scattering techniques assume that all emulsion particles are isolated and homogenous spheres. Therefore, it is not possible to differentiate the larger particles that may be formed as a result of flocculation, only diluted samples can be used in this technology for avoiding multiple scattering effects, and diluting may cause appreciable alterations in particle size distribution through the breaking of the flocculated droplets (Kartal, Unal & Otles 2017; Piorkowski & McClements 2014). Therefore, imaging with optical microscopy would provide necessary information for understanding the structure of the hydrocolloid stabilized emulsion (Hu et al. 2017; Kartal, Unal & Otles 2017). In our study, the reason for the lower particle size measurement of samples with the light scattering technique that had previously shown flocs on optical microscope images (F3.4 and F3.2) could be related to dilution and stirring steps applied in sample preparation.

TABLE 4. The particle size and zeta potential values of the samples

o:w	Sample codes	Particle size (μm)	Zeta potential (mV)
1:2	F1.2	3.74 ± 0.20^{Ba}	-29.9 ± 0.65^{Ba}
	F2.2	4.77 ± 0.58^{Aa}	-31.9 ± 0.74^{Ca}
	F3.2	2.48 ± 0.03^{Ca}	-27.5 ± 2.20^{Aa}
	F4.2	1.95 ± 0.07^{Ca}	-29.9 ± 0.86^{Ba}
1:4	F1.4	2.87 ± 0.74^{Ba}	-25.7 ± 1.22^{Aa}
	F2.4	3.05 ± 0.16^{Bb}	-30.7 ± 0.82^{Ba}
	F3.4	2.83 ± 0.17^{Ba}	$-26.5\pm0,65^{Aa}$
	F4.4	5.32 ± 0.06^{Ab}	-26.4 ± 0.81^{Aa}

Results were given as mean±SD. Different uppercase letters indicate the difference for the samples with the same oil:wall material ratio (o:w); different lowercase letters indicate the difference for the samples with different oil:wall material ratio ($p<0.05$). In each o:w ratio, from F1 to 4 RSG concentrations changed from 3 to 0% while GA increased from 0 to 3%

Characterization of Microencapsulated Flaxseed Oil Powders

The powder images were presented in Figure 4. The moisture, water activity, encapsulation efficiency (EE%), and colour values of microcapsules were presented in Table 5. The powders with more wall material (oil: wall, 1:4, w:w), had significantly higher moisture content when RSG concentration was 2.25 and 3% in the formulation, whereas at lower concentration of RSG, the oil: wall concentration did not have a significant effect on the moisture content that could be related to the possibly higher hygroscopicity of RSG. For the samples with less wall material (oil: wall, 1:2, w:w), the individual concentration of gums did not affect moisture content. All samples had similar water activity values ranging between 0.06 and 0.18. The lightness of the powders was reduced and concurrently b* value was increased in the powders with more RSG in the composition. Similarly, samples with more RSG in the composition showed lower negative a* values (greenness). While there was more oil present in the composition, the lightness value was not differently significant in most of the formulations, whereas the b value (yellowness) was significantly increased for each formulation (Table 5).

FIGURE 4. The visual appearance of microencapsulated flaxseed oil powders. 1:2 and 1:4 refers to the oil: wall material ratio of 1:2 and 1:4 (w:w). In each o:w ratio, from F1 to 4 RSG concentrations changed from 3 to 0% while GA increased from 0 to 3%

Encapsulation efficiency (EE %) of flaxseed oil was changed between 38.14 and 52.37% in freeze-dried microcapsules. It was reported that the low EE% obtained in freeze-drying could be the result of penetration of ice crystals into oil droplets and disruption of the interfacial membrane, therefore, leaking the oil from the core to the surface, leading to a higher amount of free oil in the powders (Fioramonti, Rubiolo & Santiago 2017).

In the study of Quispe-Condori, Saldaña and Temelli (2011), the EE% of flaxseed oil by freeze-drying was 32.68% and 59.63% depending on the oil: wall material ratio. Although in our study, the difference was not significant, while there was more oil in the formulation (Table 5), increasing RSG content reduced the EE % which may indicate its lower emulsifying and film-forming properties on the oil/water interface compared to

TABLE 5. The physicochemical properties of microparticles

o:w	Sample codes	Moisture content (%)	Water activity (a_w)	Encapsulation efficiency (EE %)	L*	a*	b*
1:2	F1.2	2.53±0.00^{Ab}	0.18±0.09^{Aa}	47.15± 6.02^{Aa}	81.93±0.76^{Da}	-1.69±0.02^{Aa}	24.96±0.23^{Aa}
	F2.2	2.40±0.09^{Ab}	0.15±0.09^{Aa}	39.87± 5.98^{Aa}	85.65±1.32^{Cb}	-3.16±0.08^{Ba}	20.39±0.54^{Ba}
	F3.2	2.57±0.61^{Aa}	0.10±0.07^{Aa}	50.60± 8.90^{Aa}	90.10±0.25^{Ba}	-4.19±0.05^{Ca}	18.21±0.37^{Ca}
	F4.2	2.31±0.17^{Aa}	0.11±0.08^{Aa}	52.37± 3.43^{Ab}	95.09±0.36^{Aa}	-7.66±0.04^{Db}	19.76±0.17^{Da}
1:4	F1.4	3.46±0.09^{Aa}	0.08±0.00^{Aa}	43.25± 1.91^{Ba}	82.11±0.52^{Ca}	-2.72±0.04^{Ab}	22.84±0.43^{Ab}
	F2.4	3.41±0.00^{Aa}	0.07±0.06^{Aa}	40.10± 6.21^{Ba}	87.69±0.09^{Ba}	-3.45±0.37^{Ba}	18.91±0.13^{Bb}
	F3.4	2.96±0.66^{Ba}	0.07±0.07^{Aa}	38.14± 8.14^{Ba}	88.60±1.22^{Bb}	-4.54±0.03^{Ca}	14.80±0.37^{Cb}
	F4.4	2.46±0.17^{Ca}	0.06±0.06^{Aa}	40.27± 0.42^{Bc}	93.75±1.86^{Aa}	-5.52±0.03^{Da}	10.91±0.10^{Db}

Results were given as mean±SD. Different uppercase letters indicate the difference for the samples with the same oil:wall material ratio (o:w); different lowercase letters indicate the difference for the samples with different oil :wall material ratios ($p<0.05$). In each o:w ratio, from F1 to 4 RSG concentrations changed from 3 to 0% while GA increased from 0 to 3%

GA. In the study of Perrechil et al. (2021), the rice protein concentrate (RPC) was used between modified starch for the microencapsulation of flaxseed oil by freeze-drying, the low values of encapsulation efficiency were observed when RPC concentration was increased in the formulation that was related to poor emulsifying properties of RPC compared to an excellent emulsifier, modified starch. At the same oil: wall ratio, the effect of wall material ingredients (RSG or GA) on EE % was not significant, and while with the same wall material composition, the differences were observed in the samples containing only GA, between F4.2 and F4.4, the higher EE % of flaxseed oil was observed in the higher oil: wall ratio. It was previously reported that as the emulsification capacity of the wall material increased, the migration of the oil to the capsule surface decreased (Chranioti & Tzia 2014), and many studies have shown that, the emulsions with lower particle size and increased stability results in the greater retention of encapsulated compounds (Carneiro et al. 2013; Tonon et al. 2012). Furthermore, Tontul and Topuz (2014) stated that the addition of wall material into emulsions more than the optimum level may have no further encapsulating effect.

Morphology

The SEM images of microcapsules showed irregular, flat, and cracked surfaces (Figure 5) that resembled the emulsion powders produced by freeze-drying

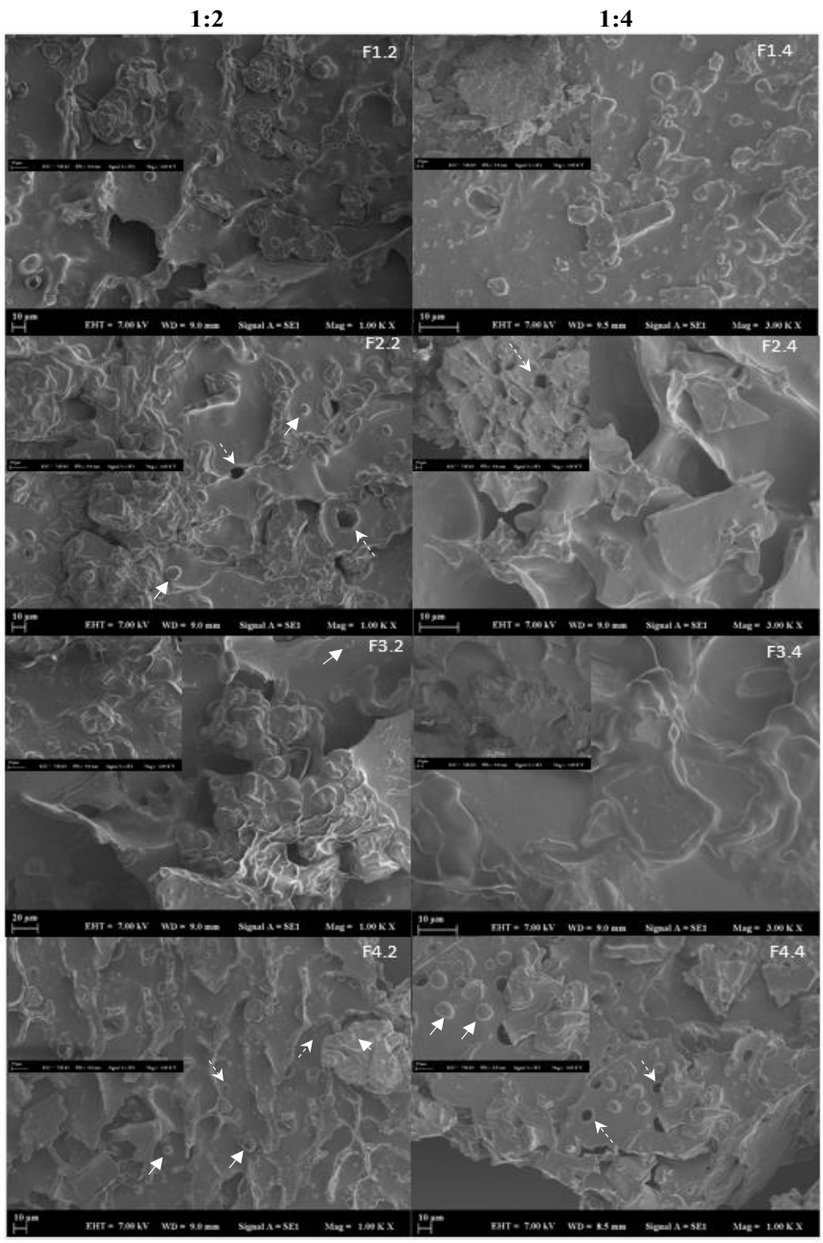

FIGURE 5. SEM images of microencapsulated flaxseed oil powders. 1:2 and 1:4 refers to oil: wall material ratio of 1:2 and 1:4 (w:w). In each o:w ratio, from F1 to 4 RSG concentrations changed from 3 to 0% while GA increased from 0 to 3%

(Fioramonti, Rubiolo & Santiago 2017; Ogrodowska, Tańska & Brandt 2017). The round-shaped microcapsules (white arrows) embedded can be seen in all samples but were more obvious in the samples produced only with GA since having RSG in the formulation created more agglomerated structures. By comparing the powders with 1:2 and 1:4 oil: wall material ratio, in all wall material formulations, the presence of agglomerated structures was less observed when there was more wall material (1:4). The air voids (dashed arrows) were also more observed in samples produced only with GA. The formation of holes in the powders is probably caused by destabilization of the emulsion during the freezing step and their formation might be related to the ice sublimation where ice would be replaced by air (Fioramonti, Rubiolo & Santiago 2017). The porous structure of freeze-dried emulsions were also determined in many previous studies (Anwar & Kunz 2011; Chranioti & Tzia 2014; Kouamé et al. 2021).

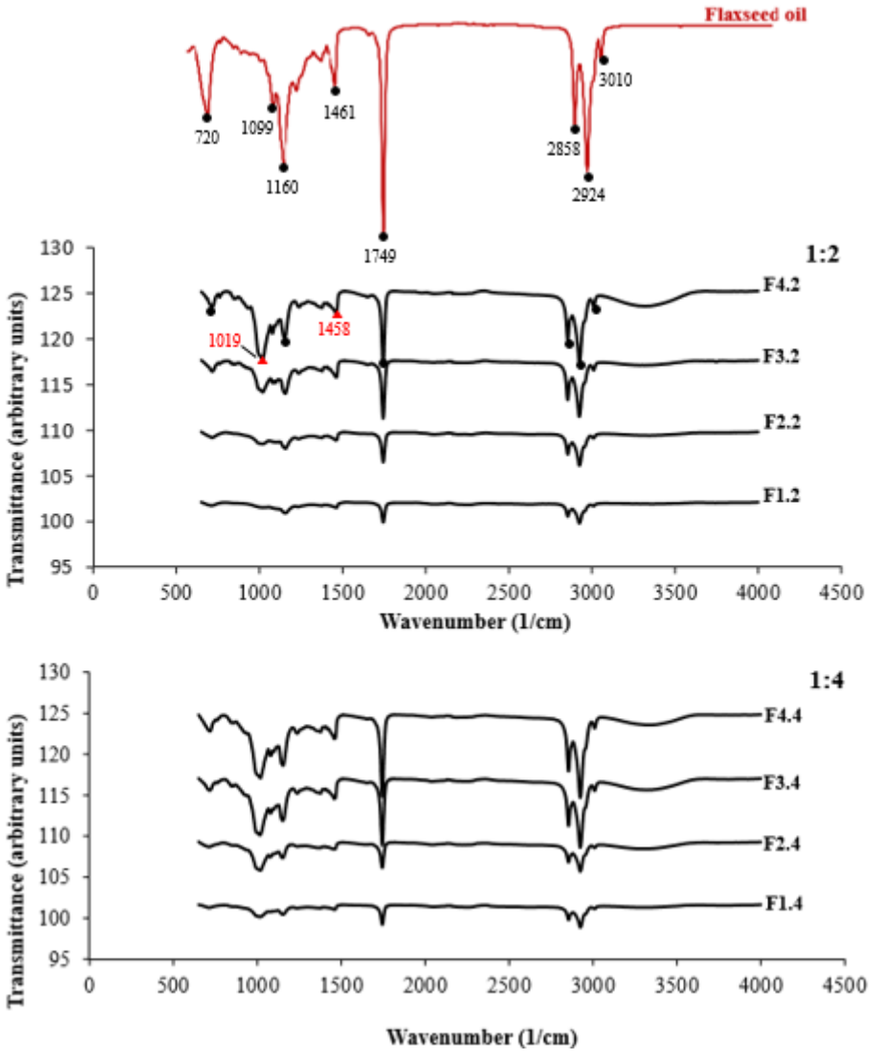

FIGURE 6. FTIR spectra of microencapsulated flaxseed oil powders. 1:2 and 1:4 refers to the oil: wall material ratio of 1:2 and 1:4 (w:w). In each o:w ratio, from F1 to 4 RSG concentrations changed from 3 to 0% while GA increased from 0 to 3%

FTIR Spectra

The FTIR spectra of flaxseed oil (Figure 6) showed characteristic CH3 and CH2 stretching peaks at 2924 cm^{-1} and 2858 cm^{-1}, and at 3010 cm^{-1} related to the bonding of = C-H from unsaturated fatty acyl chains (Mohseni & Goli 2019). The strong and sharp peak at 1749 cm^{-1} in flaxseed oil is attributed to the C=O of the ester linkages, and the absorption band at 1160 cm^{-1} and 720 cm^{-1} might refer to C-O stretching and C-H vibrations (Ozen, Weiss & Mauer 2003). It was reported by de Barros Fernandes et al. (2016) that the bands around 1019 cm^{-1} and 1458 cm^{-1} were the characteristic peaks of C-O stretching and C-H bending observed in gum arabic. In our samples, the disappearance of the bands related to GA can be observed from formulation F4 to F1, in which wall material included the highest and the lowest amount of GA. In terms of flaxseed oil encapsulation, all samples presented the peaks related to oil. In each oil: wall formulation the peaks related to oil got smaller when the RSG content was increased and GA content was reduced. The individual formulations of each oil: wall ratio had the same initial amount of flaxseed oil loading in the beginning, as a result of emulsion formation and freeze-drying, some of them stayed on the surface while some of them resided in the core of microcapsules. In both the samples with 1:2 and 1:4, oil: wall formulations followed the same trend related to RSG-GA content and flax-seed oil-related peaks. Our results confirmed that flaxseed oil was successfully encapsulated into freeze-dried powder samples.

Oxidative Stability of Microencapsulated Flaxseed Oil

Flaxseed oil has rich in polyunsaturated fatty acids, especially linolenic acid, therefore, it is susceptible to oxidation reactions when exposed to atmospheric oxygen and any thermal treatments. IP values obtained from the Oxitest analysis were used in the evaluation of the oxidative stability of the samples. IP value of the nonencapsulated flaxseed oil (control) was 3.02 h, and the IP values of the microcapsules were determined between 3.06 - 9.44 h for the samples with oil to wall ratio of 1: 2, and 4.34 - 17: 32 h for the samples with oil to wall ratio of 1: 4, respectively (Table 6). The oxidative stability of the flaxseed oil was significantly increased by the encapsulation process. Compared to free oil, the higher oxidative stability of flaxseed oil by the use of different encapsulation agents was also reported in previously published studies (Hadad & Goli 2019; Karaca, Nickerson & Low 2013; Kaushik et al. 2016). The increased oxidative stability of microencapsulated oil could be explained by the creation of a protective layer for the oil in the core of the microcapsules, which hindered the exposure of oil to the atmospheric oxygen (Goyal et al. 2015). The effect of the oil to wall material ratio and wall material types on the IP value was found to be significant ($p<0.05$). As the RSG ratio increased in wall material composition, IP values of the encapsulated samples significantly increased, indicating that RSG provided better protection from oxidation than GA and GA/RSG combination. In microcapsules produced by RSG, the IP value of encapsulated oil was increased by 3.12 and 5.73 times of the control oil in 1:2 and 1:4 oil: wall material ratio, respectively, indicating that encapsulation of flaxseed oil by RSG may provide considerable protection against atmospheric oxidation.

TABLE 6. The IP values of the microencapsulated flaxseed oils

o:w	Sample codes	IP control^{+}=3.02
1:2	F1.2	9.44^{Ab}
	F2.2	7.21^{Bb}
	F3.2	5.29^{Cb}
	F4.2	3.06^{Db}
1:4	F1.4	17.32^{Aa}
	F2.4	15.11^{Ba}
	F3.4	5.42^{Ca}
	F4.4	4.34^{Da}

$^{+}$control: free-non encapsulated flaxseed oil. Results were given as mean±SD. Different uppercase letters indicate the difference for the samples with the same oil:wall material ratio (o:w); different lowercase letters indicate the difference for the samples with different oil:wall material ratios ($p<0.05$). In each o:w ratio, from F1 to 4 RSG concentrations changed from 3 to 0% while GA increased from 0 to 3%

Compared to free oil, the higher oxidative protection of the RSG microcapsules could be related to the entrapment of oil in microcapsules preventing the contact with atmospheric oxygen and also the existence of some compounds with antioxidant properties such as protein and phenolic compounds (García-Moreno et al. 2016; Goyal et al. 2015). In our previous study, the protein (23.1, w/w), total phenolic content (17.8 mg GAE/g), and CUPRAC antioxidant activity value (148.49 mg TE/g) of RSG gum were determined (Akcicek et al. 2021). The powders produced only with GA showed the shortest IP value, thus provided limited protection from oxidation. The lower oxidative protection of the GA was also reported by Tonon et al. (2012). They reported that the flaxseed oil encapsulated with whey protein concentrate showed better oxidative stability than the samples encapsulated with GA, despite the lower encapsulation efficiency provided by whey protein concentrate. The lower IP value could be attributed to the porous structure of GA microcapsules, and those porous surfaces on the GA microcapsules could facilitate the oxygen diffusion from the air that would accelerate lipid oxidation (Jimenez, García & Beristain 2006). The results of the Oxitest suggested that RSG could be suggested as an novel encapsulating agent to improve the stability of flaxseed oil.

Conclusion

In this study, the rocket seed gum was used as an encapsulating agent for the production of microencapsulated flaxseed oil powders for the first time. The flow behaviour and dynamic rheological properties of emulsions depended on the gum arabic and rocket seed gum content, increasing the rocket seed gum provided more consistency and increased pseudoplastic characteristics of the emulsions. The droplet size of the emulsions was increased by the inclusion of rocket seed gum in the formulation probably due to its lower emulsifying property compared to gum arabic. FTIR analyzes showed more disappearance of oil related peaks when rocket seed gum content was higher that was related to residing of the oil in the core of the microcapsules. In terms of oxidative stability, the rocket seed gum provided superior protection of flaxseed oil against atmospheric oxygen compared to the both control free oil and microencapsulated oil with gum arabic alone. Our study showed that the use of rocket seed gum can be suggested as a novel encapsulation agent to improve the stability of flaxseed oil.

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