1. Introduction

Emulsion is a mixture of two immiscible solutions (usually oil and water), with one liquid dispersed into another insoluble liquid in the form of droplets. In the food industry, many products are based on emulsions, such as milk, cream, sauce and beverages. From a physicochemical point of view, emulsions are thermodynamically unstable systems that could break down over time via gravity separation, coalescence, flocculation, and phase inversion (Sriprablom, Luangpituksa, Wongkongkatap, Pongtharangkul, & Suphantharika, 2019). In practical industrial applications, emulsion stability is of great significance to its formation, production process and storage (Zhao et al., 2018). Therefore, how to produce high-quality emulsions that can maintain stability for a certain period of time is of great significance.

Animal fat can give meat good texture and unique flavor, but excessive intake of animal fat will increase the risk of cardiovascular disease, hyperlipidemia and other diseases, so reducing the amount of animal fat in meat products is imperative (Arihara, 2006; Vandendriessche, 2008). However, the processing characteristics and sensory quality of meat products will be affected by simply reducing the content of animal fat. Fat substitutes have emerged in order to ensure as many processing characteristics and quality of meat products directly is easy to produce oil precipitation, which affects the processing stability of vegetable oil has attracted extensive attention of researchers (Ansorena & Astiasarán, 2004; Ayo et al., 2007; Cáceres, García, & Selgas, 2008; Jiménez-Colmenero, Herrero, Pintado, Solas, & Ruiz-Capillas, 2010).

ABSTRACT

The effects of different concentration of flaxseed gum (FG) (0.1–0.5%, w/w) on the stability of soybean oil emulsion were studied by particle size, rheological properties, creaming stability and nuclear magnetic resonance (NMR). Results showed that emulsion particle size decreased significantly with the increase in FG concentration. Rheological measurements showed FG exhibited thickening and gelling properties. Viscosity, storage modulus, and loss modulus increased accordingly with the increase in FG concentrations, and emulsions with 0.5% FG looked like a viscoelastic solid. Emulsions with a higher FG concentration exhibited better creaming stability and structure. With the increase of FG concentration, the $^{1}$H and $^{13}$C NMR spectra line widths in high field also increased, which confirms that the interaction between FG and oil molecules is enhanced. These results show that FG can substitute for other emulsifiers or stabilizers in emulsions, and is beneficial to the stability of emulsions.

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Polysaccharides and proteins are the most commonly used emulsifiers and could control the shelf life of emulsions, many studies have confirmed this idea (Derkach, Zhabko, Voron’Ko, Maklakova, & Dyakina, 2015). The emulsifier prepared by single protein or polysaccharide has poor stability and high cost due to improper process conditions. Flaxseed gum is a natural hydrophilic colloid composed of neutral polysaccharide, acid polysaccharide and protein extracted from flaxseed. Like other hydrophilic colloids, FG has good water retention, thickening, rheological and gelation properties. It is considered that 4%~20% of FG proteins have unique emulsifying properties (Cui & Mazza, 1996; Hussain et al., 2015; Thakur, Mitra, Pal, & Rousseau, 2009). In recent years, FG is often used as a new emulsifier in food processing to improve the emulsifying stability of emulsion. Soybean oil contains linoleic acid and unsaturated fatty acid, which can reduce blood lipid and cholesterol and prevent cardiovascular and cerebrovascular diseases. Leguminous phospholipids are beneficial to the development and growth of the brain, nerves and blood vessels. Soybean oil contains palmitic acid, linoleic acid, linoleic acid, vitamin E, vitamin D, vitamin A, carotene, calcium, iron, phosphorus, lecithin and other nutrients, which has high nutritional value and therapeutic and health effects.

The emulsifying effect of FG has been preliminarily studied. Although FG has many good functions, its application in pre-emulsified vegetable oil emulsion has rarely been reported, especially on the emulsifying stability of soybean oil. Nuclear magnetic resonance (NMR), a fast and accurate technique to study emulsions, has been proven valuable, and it can be used to determine the fat and moisture content, the stability and the droplet size distribution of emulsions (Lingwood, Chandrasekera, Kolz, Fridjonsson, & Johns, 2012; Opedal, Sarland, & Sjöblom, 2010). An important advantage of NMR over other techniques is that it can be used without dilution and pretreatment of emulsions. Moreover, high-field NMR could provide information about functional groups in the form of signal peaks at different chemical shift values (Xie et al., 2016). High-field $^1$H NMR could measure different acyl groups in oil, high-field $^{13}$C NMR could provide the positional distribution information of acyl groups in oil (Zamora, Navarro, & Hidalgo, 1994). Therefore, NMR is a valuable tool in the field of emulsions.

In this study, we added different concentrations of FG to prepare emulsions and used optical images and traditional parameters, such as particle size, rheological properties, creaming index, to measure the stability of emulsions. To obtain more information, we applied NMR spectroscopy, which may allow us to gain a better understanding of the interaction between FG and oil molecules.

2. Materials and methods

2.1. Materials

FG (powder, purity 99.8%) comes from Lvqi Biotechnology Co., Ltd. (Xinjiang Province, China). Soybean oil was purchased from a local supermarket. Deuterium oxide was produced by Adamas Industrial Corporation (Shanghai, China).

2.2. Sample preparation

FG was dissolved in deionized water and mixed using a magnetic agitator to assure complete hydration. The final FG solution concentration was 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% (w/w). Then, 10% (w/w) soybean oil was added to the prepared FG solution and was homogenized using a high-speed homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany) for 2 min at 12,500 rpm. The samples were then passed through a high-pressure homogenizer at 500 MPa and 300 Mpa.

2.3. Measurement of particle size

The particle size of the emulsions was performed using a Malvern Mastersizer 3000 instrument (Malvern Instruments Ltd, Worcestershire, UK). Mean volume diameter D4,3 was used to represent the size of particles.

2.4. Rheological properties

Rheological measurements were performed on a Physica MCR 301 rheometer (Anton Paar, Graz, Austria). The frequency scanning test was carried out at 25°C to record the storage modulus (G’) and loss modulus (G”), the angular frequency range was set to 1–100 rad/s and the strain amplitude was selected as 2%. Apparent viscosity was measured by continuous shear test which was performed at 25°C over the shear rate range of 0.01–100 s$^{-1}$.

2.5. Emulsion stability

According to Meng Wang’s methods (Wang et al., 2017). The creaming stability was calculated by creaming index ($\%$) = $100 \times (H_5/H_0)$. Where $H_5$ is the height of the serum layer, $H_0$ is the total height of emulsions.

2.6. Optical microscopy

We placed a drop of emulsion on the glass sides, covered with a coverslip and then quickly put it under a 400 × optical microscope, which was equipped with a CCD camera to capture images.

2.7. High-field NMR (HF-NMR) measurements

Emulsions were prepared with D$_2$O in order to minimize the resonance of water which would interfere the information of spectrum. High-field $^1$H and $^{13}$C NMR spectra were conducted on a 400 MHz Bruker NMR spectrometer (Bruker Group, Fallanden, Switzerland) at 25°C. $^1$H NMR spectra were acquired by 16 repetitions using spectral width (SW) of 8,012 Hz and acquisition time (AQ) of 4.090 s. $^{13}$C NMR spectra were acquired by 1,024 repetitions using SW of 24,038 Hz and AQ of 1.363 s. Chemical shifts are expressed in δ units (ppm).

2.8. Statistical analysis

The data were analyzed using SAS 9.2 for windows by an analysis of variance and Duncan’s multiple range test. The
3. Resultados y discusión

3.1. Medición del tamaño de partícula

El valor de D4,3 disminuyó al aumentar la concentración de FG, pasando de 0.1% a 0.5% (Tabla 1). La disminución fue significativa (p < 0.05) en emulsiones con 0.1–0.4% FG. Sin embargo, no se encontró diferencia entre las emulsiones a concentraciones del 0.4% y del 0.5% de FG. El valor de D4,3 de emulsiones con 0.1% FG fue de 62.45 µm. Sin embargo, el valor de D4,3 de emulsiones con 0.5% FG fue de 21.08 µm, que disminuyó hasta el 66%. Estos hallazgos indican que una alta concentración de FG puede contribuir a la formación de emulsiones. Los resultados se ajustan a los resultados de estudios previos (Bai, Huan, Li, & McClements, 2017; Farshchi, Ettelaie, & Holmes, 2013; Wang et al., 2017).

FG prevenía el flocculon y la coalescencia de emulsiones adsorbiéndose en la superficie de gotas (Dickinson, 2009). Con el aumento de la concentración de FG, hay dos principales razones para el descenso del diámetro de las gotas. (i) una baja concentración de FG no fue suficiente para cubrir el área de la superficie de las gotas, y las gotas se agruparon para crear la superficie de la gota. Sin embargo, una superficie de gotas más grande se puede cubrir con una concentración suficiente de emulsionador, lo que podría cubrir superficies de gotas más rápidamente y reducir la coalescencia de gotas (Jafari, Assadpoor, He, & Bhandari, 2008). Dickinson (2009) también informó que la migración más lenta y la baja concentración de coloides no conducen a la formación de emulsiones estables.

3.2. Propiedades rheológicas

Figura 1 muestra la aparente viscosidad de emulsiones como una función de la tasa de corte (0.1-100 1/s) con diferentes concentraciones de FG. Como se esperaba, con el aumento de la tasa de corte, la aparente viscosidad del sistema disminuyó, lo que indica que las gotas exhiben un comportamiento de flujo pseudoplástico. Sin embargo, cuando aumenta la tasa de corte, el aglomerado y la coalescencia del sistema se mantienen inalterados y no se afectan por la tasa de corte. Este hallazgo fue consistente con estudios previos (Wang et al., 2017; Zhao et al., 2015). En efecto, la disminución del comportamiento de flujo se relaciona con los proporciones de fase contínua y dispersa (Lorenzo, Zaritzy, & Califano, 2008). A una menor tasa de corte, el rasgueo de moléculas de interconexión con cada gota contribuye a la formación de agregados, de tal manera, el flujo de fase contínua es relativamente fuerte, y las emulsiones exhiben un comportamiento viscoso. Con el aumento de la tasa de corte, la aggregación y el arreglo de las moléculas a lo largo del flujo disminuyeron "gradualmente destruido" (Oh, So, & Yang, 1999). La resistencia al flujo de fase contínua disminuyó con el aumento de la tasa de corte, lo que revela el comportamiento pseudoplástico. Podemos observar en Figura 1 que las muestras con mayores concentraciones de FG exhiben una mayor viscosidad. Los estudios previos han mostrado que las propiedades rheológicas de las emulsiones están estrechamente vinculadas a la estabilidad de las mismas. (Felix, Romero, & Guerrero, 2017; Hu et al., 2016). El aumento de la viscosidad y la formación de FG concentran indica que FG contribuye a la viscosidad del sistema y a la formación de una emulsión estable.

En el artículo se presentan los resultados de la viscosidad y los hallazgos que se mencionan. En la Tabla 1 se presenta la media ± desviación estándar de los valores. Los resultados muestran que a medida que aumenta la concentración de FG, la viscosidad disminuye significativamente (p < 0.05). Los valores de D4,3 disminuyeron de 62.45 ± 5.69 µm a 21.08 ± 0.95 µm, lo que indica una mejor estabilidad de las emulsiones.

| Flaxseed gum concentration (Flax gum) | D4,3 (D₄,₃) | 0.1% | 0.2% | 0.3% | 0.4% | 0.5% |
|--------------------------------------|------------|------|------|------|------|------|
|                                      | (µm)       |      |      |      |      |      |
| 0.1% FG                              | 62.45 ± 5.69<sup>A</sup> |      |      |      |      |      |
| 0.2% FG                              | 45.37 ± 2.35<sup>B</sup> |      |      |      |      |      |
| 0.3% FG                              | 38.02 ± 2.68<sup>C</sup> |      |      |      |      |      |
| 0.4% FG                              | 24.05 ± 1.99<sup>D</sup> |      |      |      |      |      |
| 0.5% FG                              | 21.08 ± 0.95<sup>D</sup> |      |      |      |      |      |

Nota: Los valores se expresan como el promedio ± desviación estándar y se consideran significativos si p < 0.05. Las letras diferentes en la misma fila indican diferencias estadísticamente significativas en p < 0.05.
frequency. Moreover, $G'$ and $G''$ in emulsions with 0.5% FG were less dependent on frequency, thereby also suggesting a stronger structure of the samples. The results further showed that the structure of emulsion could be enhanced by adding FG.

### 3.3. Creasing stability measurement

The creasing stability of the emulsions stabilized by FG was determined after 1 and 24 h of storage (Figura 3). After 1 h of storage, emulsions with FG concentration greater than 0.3% became resistant to creasing. By contrast, other emulsions were immediately separated into two phases. The creasing stability of emulsions significantly ($P < 0.05$) improved with the increase in FG concentration after 1 or 24 h of storage given that the creasing index decreased significantly ($P < 0.05$) with the addition of FG. This phenomenon indicated that FG had an obvious influence on the creasing stability of emulsions and can promote the stability of emulsions, which was in good agreement with the findings of previous researchers (Liang, Wong, Pham, & Tan, 2016; Zhao et al., 2015).

Due to the instability of the emulsion itself, it will be separated into two phases during storage, thus reducing the free energy of the system. And during the storage, many factors, such as flocculation, aggregation, Ostwald ripening may cause emulsion stratification (Dickinson, 2009; Zhu, Liu, Chen, Cheng, & Zou, 2018). However, because of its comprehensive properties, such as thickener, water retaining agent or gel agent, FG can be widely used in emulsion to improve its stability. Once oil droplets were formed, FG would quickly adsorb on the surface of oil droplets, thus preventing them from colliding with each other to form larger droplets (Dickinson, 2009). On the other hand, FG can slowdown or even prevent oil-water separation by increasing the viscoelastic properties of continuous phases. This result was consistent with the findings on higher viscosity in the addition of FG (Figura 1).

### 3.4. Optical microscopy

The microscopic images in Figura 4 show the effect of FG concentration on the structure of samples. It can be seen that emulsions with different FG concentration showed great differences in the structure. Larger particle sizes were found in lower FG concentration samples, corroborating our findings regarding particle size (Table 1). The extent of flocculation decreased as a function of FG concentration, which was previously reported by other researchers (Sun, Sun, Wei, Liu, & Zhang, 2007; Wang et al., 2017). At a lower FG concentration, there is not enough FG to cover the surface of oil droplets. Some oil droplet molecules gather together to form large particles under the action of FG, while others are separated into layers on the surface. Thus, the samples were unstable. This phenomenon showed that the concentration of FG has a significant effect on the emulsion. This condition could be explained by the depletion flocculation caused by non-adsorbing FG, which resulted in enhanced serum separation of emulsions (Dickinson, 2009). By contrast, the emulsions with a higher FG concentration had a smaller particle size, because sufficient FG was available to wrap the surface of oil droplets, thereby causing the samples to disperse uniformly and have higher stability.

### 3.5. HF $^1$H and $^{13}$C NMR measurements

In the past decades, high-field $^1$H and $^{13}$C NMR spectroscopy had been widely used to study the structural, spatial, and electronic structures of individual organic compounds (Kononova et al., 2017; Rakhmatullin et al., 2015). In fact, NMR spectroscopy can also provide a broad opportunity to study the structure of oil dispersions and determine their physicochemical properties. It can measure different acyl groups in oils and fats, and provide useful information for the analysis of emulsion structure (Guillén & Ruiz, 2010). It is also a powerful tool for studying the mobility of oil droplets. In our emulsions, considering the higher mobilities of oil droplet molecules in the aqueous phase and at the interface compared with FG, the oil signal was thought to be the dominant signal in the NMR spectra (Day, Xu, Hoobin, Burgar, & Augustin, 2007).
Figura 5 shows the high-field $^1$H NMR spectra distribution of samples with 0.1–0.5% FG. As we can see, samples with different FG concentrations exhibited the same resonance patterns but varying line widths, suggesting that the interaction between oil and FG at the oil–water interface was weaker than that of oil molecules, which means interaction between oil molecules played a major role in the signals. According to previous studies, the increase in line width with increased FG concentration showed a stronger interaction between oil and FG molecules (Day et al., 2007). In addition, the increase in line width reduced the mobility of oil molecules, which may be attributed to the increased interaction between oil molecules and FG (Day et al., 2007; Wang et al., 2017). Previous researches have extensively confirmed the chemical shifts of triglycerides in high-field $^1$H NMR (Fernández, Hilty, Wider, & Wüthrich, 2002; Liu et al., 2017). The chemical shifts obtained in HF $^1$H NMR for the groups on the oil molecules are shown in Table 2. As seen from the table, -(CH$_2$)$_n$-group had more freedom of mobility, which suggested it was further away from the interface and had more space to stretch.

High-field $^{13}$C NMR can be used to confirm the acyl positional distribution of oils, which was superior to the high-field $^1$H NMR (R. Zamora et al., 1994). Figura 6 shows the $^{13}$C NMR spectra of samples with different FG concentrations. As we can see, emulsions with 0.1% FG displayed weak $^{13}$C spectra, and many signal peaks were not scanned. This phenomenon may be due to the poor stability of emulsions. In contrast, samples with 0.2–0.5% FG displayed strong $^{13}$C spectra and all of them appeared similar, with the differences being seen only in the line width and height of peak. For a certain peak, the linewidth was positively correlated with FG concentration. For example, when the chemical shift is 22.53 ppm, the linewidth of samples containing 0.2%-0.5% FG is 5.42, 7.24, 8.53 and 9.72, respectively. This finding was consistent with the results observed in the high-field $^1$H NMR spectra, indicating the increased interaction between FG and oil molecules with increased FG concentration. Moreover, the emulsions with higher FG concentration displayed a smoother baseline in the spectra, thereby potentially serving as a reference to evaluate the stability of emulsions.

Figure 4. Microscopic images of emulsions containing (a) 0.1%, (b) 0.2%, (c) 0.3%, (d) 0.4%, and (e) 0.5% FG.
Figura 4. Imágenes microscópicas de emulsiones que contienen (a) 0.1%, (b) 0.2%, (c) 0.3%, (d) 0.4% y (e) 0.5% FG.
**Figure 5.** The high-field (HF) $^1$H NMR spectra of the emulsions in D$_2$O containing 10% soybean oil with different FG concentration of: (a) 0.1%, (b) 0.2%, (c) 0.3%, (d) 0.4%, (e) 0.5%.

**Tabla 2.** Desplazamiento químico obtenido en HF $^1$H RMN para los grupos de las moléculas de aceite.

| Signal | Chemical shift (ppm) | Functional group          |
|--------|----------------------|---------------------------|
| 1      | 0.78                 | -CH$_3$ (acyl group)      |
| 2      | 1.19                 | -(CH$_2$)$_n$- (acyl group)|
| 3      | 1.47                 | =OCO-CH$_2$-CH$_2$- (acyl group)|
| 4      | 1.92                 | =CH$_2$-CH = CH- (acyl group)|
| 5      | 2.14                 | =OCO-CH$_2$- (acyl group)|
| 6      | 2.65                 | =HC-CH$_2$-CH = (acyl group)|
| 7      | 3.98                 | -CH$_2$OCOR (acyl group)|
| 8      | 4.17                 | -CH$_2$OCOR (glyceryl group)|
| 9      | 4.70                 | Water                     |
| 10     | 5.18                 | -CH = CH- (acyl group)    |
4. Conclusions

In our research, FG has been proved to function as an effective stabilizer for oil-in-water emulsions. D4,3 decreased significantly with the increase in FG concentration, indicating that a higher FG concentration could improve the stability of emulsions. As for apparent viscosity, all samples exhibited a typical shear thinning flow behavior. Similar to apparent viscosity, G’ and G” showed an uptrend with the increase in FG, and samples with 0.5% FG behaved like a viscoelastic solid. The creaming stability of emulsions improved with the increase in FG concentration. Microscopic images also confirmed that higher FG could form a more stable emulsion. High-field NMR spectra provided information on the mobility of oil molecules and the interaction between oil and FG at the interface. With the increase in FG concentration, the line widths in the high-field $^1$H and $^{13}$C NMR spectra increased, confirming the increased interaction between FG and oil molecules. In addition, -(CH$_2$)$_n$- was confirmed as the most mobile group and furthest away from the oil/FG interface. These findings indicate that FG can stabilize emulsions and that a 0.05% concentration corresponds to better stability of emulsions.

Disclosure statement

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