Original Research

Rheology and physical properties of sonicated chickpea concentrate with fructans in aqueous media and stable emulsions

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Abstract: Based on the rheological and other physical properties of chickpea concentrate (CPC)/fructan aqueous mixtures containing 0.2% carboxymethyl cellulose (CMC) and their respective emulsions, the macrostructure of the system was elucidated, and its effect on the stability of the proposed emulsions was evaluated. The zeta potential and particle size of the CPC dispersions subjected to ultrasonic treatment were analyzed. Neither agave (AGF) nor chicory fructans (CHF) affected the rheological behavior (Newtonian) of aqueous systems containing only CPC. A Carreau behavior was observed when CMC was added. A synergistic effect occurred when 6% CHF was mixed with CMC. Furthermore, CHF led to a higher overall viscosity than AGF in the systems studied. Fructans changed the total charge of the protein dispersion and increased the particle size. The viscoelastic properties confirmed the liquidlike behavior of the aqueous mixtures. Emulsions containing CPC/CMC mixtures and fructans exhibited thixotropic behavior; emulsions containing 30% oil followed the Carreau model, while emulsions containing 60% oil were fitted to the Hershel-Bulkley model for upward and downward shear rate curves. The latter behavior corresponded to the formation of a weak gel structure and a flocculated oil droplet network, as confirmed by the mechanical spectra. The droplet size in the emulsions decreased in the presence of fructans, particularly for 3% CHF and 6% AGF. However, 6% CHF led to the formation of the most kinetically stable emulsions. These results confirm the possibility of incorporating CPC and fructans as oil-in-water emulsions stabilized with CMC into healthy plant-based products.

Keywords: Chickpea concentrate, Fructans, Carboxy methylcellulose, Emulsion, Rheology, Physical properties

1. Introduction

 With the increasing worldwide demand for healthy and sustainable foods, there is a new and growing need for the development of functional foods that contain prebiotics, such as agave or chicory fructans and plant-based proteins. Although fructans have many applications in dairy products and baked goods [1, 2], the combination of plant-based proteins with fructans has not yet been tested in emulsions used in the food industry to deliver nutrients. Peas, chickpeas and lentils are legumes that have attracted interest due to the sustainability of their production [3]. Nevertheless, the functional properties of these proteins are not comparable to those of milk proteins. Studies are

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being conducted to improve the emulsifying and foaming properties of legume proteins and different methods, such as pH modification, heat treatment, high-pressure treatment and ultrasonic (US) treatment have been applied, either alone or in combination [4, 5].

 Chickpea is a legume that provides carbohydrates, proteins, dietary fibers, vitamins, minerals and bioactive compounds [6]. It has a wide range of applications due to its high yield and low cost. Chickpea proteins are considered to be of higher quality than the proteins produced by other pulses and contain mainly globulin and albumin, which account for 42.17% and 39.76% of the total protein, respectively [7]. However, the application of chickpea proteins as food ingredients is still in its beginnings and their properties and interactions within food matrices are scarcely studied [8]. In food applications, isolated chickpea proteins (ICPs) have ideal emulsifying properties at appropriate ionic strengths and pH values [9]; nevertheless, the industrial production of ICP is still incipient. Chickpea concentrate (CPC) without a high degree of refinement is now more widely marketed, with the advantage preserving other nutrients, such as minerals, dietary fibers, and lipids. Products developed that include CPC could be more accepted by athletes or vegetarian consumers, who are looking for plant-based nutrients.

 The findings of previous studies indicated that US increases the surface hydrophobicity of proteins by breaking the non-covalent bonds between protein chains, resulting in smaller and more evenly dispersed protein aggregates that alter functional properties [4,10]. In this context, the CPC in this research was subjected to constant US irradiation, which was chosen in preliminary tests to obtain a more ordered structure, and the hydrophobic and charged groups originally buried inside were exposed to improve the surface properties. In addition, the application of ultrasound is an effective pretreatment method for reducing the hydration time [11].

 Oil-in-water emulsions comprise immiscible oil droplets dispersed in water, a thermodynamically unstable system that tends to break down over time because of various mechanisms, including gravitational separation, flocculation, coalescence, particle coalescence, Ostwald ripening and phase separation [12]. The rheological behavior of these materials depends on their composition, structure, interactions and processing [13]. Under flow conditions, Newtonian and non-Newtonian shear thinning behavior and yield stress can be observed, depending on the oil concentration and type of thickener and emulsifier used.

 Recently, it was reported that low-concentration emulsions (3.2% oil) containing a commercial gelling CPC (3.5% w/v) showed Newtonian behavior and low viscosity. In addition, when 0.1 mM NaCl was added to the system, shear thinning behavior was also observed. When both treatments were applied (salt and heat treatment), a yield stress developed, resulting in Herschel-Bulkley behavior. This characteristic was also observed in samples where a standard commercial CPC (non-gelling) was used, confirming that the rheology of the emulsions depends completely on the formulation and the processing applied [14]. Furthermore, when the aqueous phase exhibits non-Newtonian behavior due to the thickener used, the emulsion may show the same rheological behavior or be more complicated, depending on the interactions that occur between the oil droplets and the macromolecules present in the emulsion [15]. For example, emulsions containing polysaccharides such as galactomannans, xanthan gum or mixtures of polysaccharides showed weak gel-like rheological behavior (viscoelastic properties), and non-Newtonian shear-thinning behavior and yield stress were observed in sonicated emulsions under flow conditions [16, 17].

 The use of polysaccharides to prevent the aggregation of proteins has been proposed to improve protein stability due to their thickening effect [18]. Nevertheless, the thermodynamic incompatibility between polysaccharides and proteins or the formation of protein/polysaccharide coacervates needs to be understood [19, 20]. In this study, we chose carboxymethyl cellulose (CMC), which is commonly used as a stabilizer in emulsions, such as in milk drinks, and is considered one of the most important anionic water-soluble polysaccharides. In addition to its compatibility with the proposed system, it is composed entirely of a backbone of *β* (1→4)-linked glucopyranose repeating units that may interact with positively charged proteins. The addition of CMC to food-dispersed systems increases the viscosity of the continuous phase and results in the formation of an entangled polymeric network [21]. However, the presence of short-chain polysaccharides such as fructans could affect macromolecular interactions and associations in food-dispersed systems, which mainly depend on their structure. For chicory fructans (CHFs), the fructose molecules are mainly linear β (2→1) with 2-60 degrees of polymerization [22], while those of agave fructans (AGFs) are branched β (2→1) and β (2→6), with 3-29 degrees of polymerization [23].

 The objective of this study was to analyze the rheological behavior (flow and viscoelastic properties) and physical properties of CPC/fructan aqueous mixtures in the presence of CMC as well as fructans with or without CMC and to evaluate their impact on the production of stable emulsions for the development of functional food alternatives. The viscosity was analyzed as a function of shear rate; the mechanical spectrum, zeta potential and particle size of the CPC/fructan aqueous mixtures were measured. The particle size and stability of the emulsions were evaluated and quantified.

2. Materials and methods

2.1 Materials

 Commercial CPC contains 68% protein, 23% lipids, 3% carbohydrates and 5.5% water (HSN, Granada, Spain); commercial fructans, AGF with 96.3% carbohydrates, corresponding to 89.17% dietary fiber and 7.13% sugars, and 3.7% water (Mieles Campos Azules, Amatitán, Mexico); CHF with 97% carbohydrates, corresponding to 94.09% dietary fiber and 2.91% sugars, and 3.0% water (ebien, Querétaro, Mexico); CMC, Cekol 1000000, high viscosity (Ingredion Mexico, Tlalnepantla, Mexico); commercial safflower oil, rich in linoleic and oleic acids (Oléico, San Luis Potosí, Mexico) [24] ; and purified water (e-Pura, The Pepsi Bottling Group Mexico, Ciudad de México, Mexico). Sodium azide (Hycel de México, Zapopan, Mexico) was used as an antibacterial agent (0.03%) .

2.2 Methods

2.2.1 Sample preparation

 The samples were prepared considering the moisture content of the powders. CPC was slowly added to water (23-25 °C) and mixed with a propeller stirrer at 360 rpm for 45 min (RW20, IKA Werke GmbH & Co., Stiffen im Breisgau, Germany); CMC was prepared under the same conditions. AGF or CHF powder was incorporated into water (23-25 °C) with constant stirring for 1 hour (magnetic stirrer, Barnstead International, Iowa, USA).

 To enhance their emulsifying properties, the CPC dispersions were sonicated with a sonotrode S24d22D (UP400ST, Hielscher Ultrasonics, Teltow, Germany) at 24 kHz, 90% amplitude and 50% pulse for 7 minutes at 100 mL $(\sim 53 \text{ kJ/L})$. An ice bath was used to prevent the samples from reaching 50 °C.

 Mixtures were prepared as described in a previous study by mixing equal volumes of aqueous solutions with twice the required concentration of each component using the same magnetic stirrer for 30 minutes. The samples were homogenized for 1.5 minutes (2000 rpm, L4R, Silverson Machines, Chesham, UK) and refrigerated overnight (~4 °C) [25]. In the aqueous systems tested, 0.2% CMC was used as a thickener, the CPC was at 4% concentration and pretreated with ultrasound unless otherwise indicated, and AGF and CHF were used at concentrations of 3% and 6%, respectively.

2.2.2 Formation of emulsions

 Appropriate quantities of oil were added to the protein/ polysaccharide aqueous mixtures at room temperature (~23 °C) to generate oil-in-water emulsions with 30% or 60% oil. High-speed lab-scale production of emulsions was conducted at 7000 rpm for 90 s (L4R), followed by 15000 rpm for 90 s (30% oil emulsions) or 20000 rpm for 120 s (60% oil emulsions) (T25 Easy Clean Ultraturrax, IKA Werke GmbH & Co.).

2.2.3 Flow properties

 The flow curves of the low-viscosity systems were determined with a controlled shear stress rheometer (MCR301, Anton Paar, Graz, Austria) at 25 °C using a cone–plate system (CP75-1) with a cone of 75 mm diameter, a 0.149 m truncation and an angle of 1°. A shear rate ranging from 100 to 400 $s⁻¹$ was applied. The flow curves of more viscous systems were determined with a controlled shear rate rheometer (Rheolab QC, Anton Paar) at the same temperature using a double-gap system (DG42) with a radius ratio of 1.0246 and an effective measuring gap length of 78.70 mm. A shear rate of 10 to 500 s^{-1} was applied. All the rheometers were equipped with a temperature control unit (Peltier). Some experimental data were adjusted to Newton's law of viscosity (Eq. 1), where the slope of the straight line of shear stress (σ_{yx}) as a function of the shear rate ($\dot{\gamma}_{vx}$) corresponds to the shear viscosity (*η*) of the liquid.

$$
\sigma_{yx} = \eta \, \dot{\gamma}_{yx} \qquad (1)
$$

 Other aqueous mixtures were adjusted to the Ostwald– de Waele model using a power function (best-fit curve, Excel), which is characterized by two parameters, the consistency index (k) and the flow behavior index (n) .

$$
\sigma_{yx} = k \dot{\gamma}_{yx}^{\quad n} \tag{2}
$$

 Fluids containing CMC were adjusted to the Carreau model (Eq. 3) (Rheoplus software, version 3.61). This model includes the constant viscosity at low shear rates (η_0) , called the zero-shear viscosity, a characteristic time (λ) and an exponent (p) related to the shear-thinning behavior. The constant viscosity evaluated at high shear rates (η_{∞}) , included in the model, was negligible as the computed data were less than the viscosity of water.

$$
\eta = \eta_{\infty} + (\eta_0 - \eta_{\infty}) \Big[1 + \big[\lambda \big(\dot{\gamma}_{yx} \big) \big]^2 \Big]^{-p} \tag{3}
$$

 The concentrated emulsions (60% oil) were adjusted to the Herschel–Bulkley model (Eq. 4) (Rheoplus software, version 3.61). After exceeding a yield stress (σ_0), these fluids behave as shear-thinning materials such as Ostwald– de Waele fluids and are characterized by a consistency index (k) and a flow behavior index (n) .

$$
\sigma = \sigma_0 + k(\dot{y}_{yx})^n \tag{4}
$$

2.2.4 Viscoelastic properties

 The viscoelastic properties were determined with a controlled shear stress rheometer (MCR92, Anton Paar) at 25 °C using concentric cylinder systems (CC27 and CC39, with a radius ratio of 1.0846 and 1.0848, and gap lengths of 39.998 and 60.001 mm, respectively). The linear viscoelastic range was determined by performing a strain sweep at a constant angular frequency (*ω*, 10 rad/s) over a strain range from 0.5% to 10% for aqueous samples and 0.1% to 1% and 0.01% to 0.5% for emulsions with 30% and 60% oil, respectively. The dynamic oscillatory test was performed over an angular frequency range of 0.5-10 rad/s for aqueous samples and 0.5-50 rad/s for emulsions. The storage modulus (G') and loss modulus (G'') as a function of angular frequency were evaluated at controlled strains of 0.75%, 0.25% and 0.05% for aqueous samples and emulsions with 30% and 60% oil, respectively. The experimental and were adjusted using the following equations:

$$
G' = \alpha' \omega^{\beta} \qquad (5)
$$

$$
G'' = \alpha'' \omega^{\beta''} \qquad (6)
$$

where α' , β' , α'' , and β'' are experimentally determined constants.

2.2.5 Particle size and zeta potential measurements of the aqueous samples

 The particle size and zeta potential of the CPC/fructan aqueous mixtures and CPC alone were measured using dynamic light scattering (DLS) and electrophoretic mobility, respectively (Zetasizer Nano-ZS90, Malvern Instruments, USA). The measurements were performed after diluting the samples in water. All measurements were performed in triplicate with freshly prepared samples at a constant temperature of 20 °C and an angle of 90°. A refractive index (RI) of 1.450 was used for each protein sample, and water with a viscosity of 0.8872 mPa s was used as the dispersant . The pH value (Oakton PH700, Singapore) was subsequently measured.

2.2.6 Particle size distribution of emulsions

 The droplet size distribution of the emulsions was measured after emulsification with a particle size analyzer (930 L/D, CILAS, Orleans, France) at room temperature. According to lSO13320:2009, a few drops of the emulsion samples were successively added to a tank filled with water (\sim 400 mL) until an obscuration of 5-10% was reached. The particle analysis was based on the diffraction of a light source by the samples under examination using Fraunhöfer theory [26] in a particle size range from 0.2 to 500 m in wet dispersion mode (CILAS, Orleans, France).

 To avoid the contribution of flocculated droplets, the test was conducted by applying US during the measurement (55 kHz) under constant stirring. The distribution curve

was represented by 100 classes over the measurement range, and the volume-weighted mean diameter (D_4) was determined by Eq. (7) (SizeExpert V9.08 software); the relative span (RS) was estimated using Eq. (8). $D_{0.1}$, $D_{0.5}$ and $D_{0.9}$ indicate the droplet sizes at which 10%, 50%, and 90% of the distribution was smaller than the droplet size, respectively [27].

$$
D_{4,3} = \sum n_i D_i^4 / \sum n_i D_i^3
$$
 (7)

$$
RS = (D_{0.9} - D_{0.1}) / D_{0.5}
$$
 (8)

2.2.7 Emulsion stability

 The stability of the emulsions stored in the refrigerator was measured with a Turbiscan MA2000 (Formulaction, Toulouse, France) by transmission (T) and backscattered (BS) light through the sample, every 40 m. Briefly, fresh emulsions were placed into flat-bottomed cylindrical glass tubes (6 mL), and measurements were performed at different times after the emulsions were prepared; samples were kept under refrigeration $(4 \degree C)$, and several readings were taken after conditioning at room temperature for approximately 7 days. The curve profile obtained was interpreted in raw mode, taking only the backscattered light (expressed as a percentage) versus height. For the reference mode, BS profiles at $t = 0$ min were used as baseline and were subtracted from the profiles at other time points to obtain ∆BS (Turbisoft V1.2.1 software).

2.3 Statistical analysis

All sample data were reported as the mean \pm standard deviation. One-way analysis of variance (ANOVA) with Tukey's comparison was used to determine significant differences between the fructan sources at the 5% significance level (Minitab 17, Minitab, Inc., USA). All the measurements were conducted in triplicate.

3. Results and discussion

3.1 Flow properties of chickpea concentrate/ fructan aqueous mixtures

 The observed Newtonian behavior of the CPC dispersion was similar to that of aqueous ICP dispersions, which are Newtonian fluids with a protein concentration of up to 4% [28]. In this study, the CPC dispersion exhibited a higher shear viscosity than the CHF solutions (3% and 6%) (Table 1). This behavior was due to the higher molecular mass and the larger hydrodynamic radius of the proteins in the CPC dispersion. At the same concentrations as CHF, AGFs are likely to develop a lower viscosity than CHFs because they have a lower intrinsic viscosity [29].

Table 1. Viscosity and Ostwald-de Waele parameters of CHF solutions, CPC dispersion and aqueous mixtures with fructans, 25 °C.

Agave fructans (AGF), chicory fructans (CHF), and chickpea protein concentrate (CPC, 4%). The concentrations, expressed as percentages, are represented by 3 and 6. Tukey comparison by group and column, 95% confidence interval. Means that do not share a letter are significantly different.

 CPC/AGF aqueous mixtures, such as liquid materials with low molecular weight soluble compounds or low particle concentrations that do not exceed colloidal size (< 10 *μ*m), also exhibit Newtonian behavior (Eq. 1) [15]. Statistically significant differences in shear viscosity were found for the CHF, CPC and CPC/AGF aqueous mixtures $(p < 0.05)$ (Table 1).

 Mixtures with CHFs exhibited higher viscosity and shear-thinning behavior under shear. The flow curve was fitted to the Ostwald-de Waele model (Eq. 2), where the behavior indices and the consistency indices of the mixtures with 3% and 6% CHF differed significantly ($p <$ 0.05) (Table 1). The consistency indices obtained imply a higher global viscosity than in the mixtures with AGF; the flow behavior indices indicate that the viscosity decreases slightly due to shearing.

3.2 Zeta potential and particle size of chickpea concentrate/fructan aqueous mixtures

 The CPC dispersions showed a negative zeta potential value (−15.03 ± 0.29 mV) at the native pH (pH 5.9). For CPI dispersed in deionized water (0.1 mg/ mL), the dispersions without US had a zeta potential of -28 mV. Samples with US had a decreased absolute zeta potential as the intensity of the treatment increased, which was attributed to the fact that additional positively charged groups were exposed and neutralized some of the negative charge on protein surfaces [30].

 In addition, the lower zeta potential observed in the CPC dispersions could be due to the contribution of the other components present that counteract the charge of the protein, such as NaCl in pea proteins [31]. The lower zeta potential observed in the CPC dispersions could also be due to the pH of the medium. Considering that an isoelectric point (pI) between pH 4.5 and 5 was reported for legume proteins [32], and combined with the effect of US and the presence of other compounds, the zeta potential was justified.

 The CPC/fructan aqueous mixtures (pH 6.0-6.1) had a significantly lower ($p < 0.05$) absolute zeta potential than the samples obtained with the CPC dispersion; the mixtures with AGF had lower absolute values (-7.16 \pm 0.32 mV) than the mixtures with CHF $(-10.40 \pm 0.26$ mV). These results are probably due to the fact that AGF and CHF were weakly charged polysaccharides (-4.8 mV and -8.8 mV, respectively, at 10% w/w) [33, 34]. Similar decreases in the absolute zeta potential were found for milk protein–inulin mixtures [34], milk protein–agave fructan mixtures [33] and pea protein–corn fiber gum mixtures [18], confirming that despite the molecular size and low charge of fructans compared to CPCs, they modify the total charge of proteins.

 The average particle size of CPC aggregates in aqueous media was 166.3 ± 3.3 nm. After the addition of AGF or CHF to the CPC dispersion, the average particle size increased significantly ($p < 0.05$), with values of 213 \pm 2.1 nm for the CPC/6% AGF aqueous mixture and 313 \pm 12.2 nm for the CPC/6% CHF aqueous mixture. These results were consistent with the increase in viscosity and non-Newtonian behavior observed in the CPC/CHF aqueous mixture. The increase in the particle size of the CPC aggregates upon the addition of fructans might reflect the coating of AGF or CHF on the surface of the protein, which may be attributed to the weak hydrophobic interactions and hydrogen bonding between CPC and AGF or CHF, similar to what has been observed for pea protein mixed with weakly charged polysaccharides [18]. The differences observed between the AGF and CHF mixtures

were due to the more compact form of the AGF branched molecules in solution compared to the linear molecules in CHF [29].

3 . 3 F l o w p r o p e r t i e s o f t h e carboxymethylcellulose/fructan aqueous mixtures

 As a high-molecular-mass biopolymer, the CMC solution exhibited a constant viscosity at low shear rates (plateau) and a shear-thinning behavior at high shear rates, as previously reported [35]. Figure 1 shows the viscosity as a function of the shear rate of the CMC solution and the aqueous mixtures. The overall viscosity of the binary mixtures was greater than that of the CMC solution, mainly at shear rates greater than $100 s⁻¹$.

Figure 1. Viscosity as a function of the shear rate of the CMC solution and aqueous mixtures at 25 °C. Nomenclature as in Table 1 and Table 2.

 A mixture of CMC with 6% CHF exhibited the highest viscosity over the entire range of shear rates. A synergistic effect was observed, in which the overall viscosity of the mixture was greater than the sum of the individual viscosities. This synergism may be attributed to the formation of interpolymer complexes between both biopolymers, as reported for mixed hydroxypropyl- and carboxymethyl hydroxypropyl-substituted guar gums, as confirmed by UV spectroscopy measurements [36].

3.4 Flow properties of chickpea concentrate/ carboxymethylcellulose aqueous mixtures

 In contrast, the CPC/CMC aqueous mixture presented the lowest viscosity at shear rates below 100 $s⁻¹$, which was even lower than that of the CMC solution, probably due to the interference of protein molecules between CMC molecules (Figure 1). The viscosity shear-thinning curves of the CMC solution and the aqueous mixtures were characteristic of the Carreau model (Eq. 3, $R^2 > 0.99$). Table 2 summarizes the Carreau parameters; η_0 , λ and p . The zero-shear viscosity of the CMC/6% CHF mixture

(0.146 Pa s), was 1.92 times greater than that of the CMC solution, confirming the synergistic effect, followed by the CPC/CMC/6% CHF aqueous mixture (0.101 Pa s), which significantly differed ($p < 0.05$).

 The CPC/CMC aqueous mixture exhibited the lowest η_0 and Carreau parameters; these values were lower than those of the CMC solution (0.2%), even though the CPC concentration was 4% . The decrease in η_0 and rheological parameters is due to a decrease in intermolecular interactions between the entangled CMC molecules caused by the interference from aggregated protein macromolecules. However, the decrease in η_0 of the CMC solution with the addition of CPC was only 21%.

3.5 Flow properties of chickpea concentrate/ carboxymethylcellulose/fructan aqueous mixtures

 As for the viscosity of the CPC/fructan aqueous mixtures, the global viscosity of the mixtures with three components increased because of the presence of fructans, and the highest viscosity observed was 6% CHF (Figure

1). Nevertheless, the overall viscosity was lower than that of the CMC/6% CHF binary mixture at shear rates lower than $100 s^{-1}$.

 When fructans were added to the CPC/CMC aqueous mixtures, η_0 increased with the fructan concentration. No significant differences in η_0 were observed between the CPC/CMC/3% CHF and AGF aqueous mixtures and the CMC solution. CHF increased η_0 more than AGF, as observed from the Newtonian behavior of the CPC/ fructan aqueous mixtures, which was caused by the hydrodynamic radius of straight-chain CHF being greater than the radius of branched AGF. In addition, the degree of polymerization associated with CHF [22] might be greater than that associated with AGF [23], contributing to the increase in η_0 .

 The addition of fructans modified the Carreau parameter *λ*, which is related to the onset of shear-thinning behavior; *λ* increased from 0.026 to 0.052 s, indicating that the onset is shifted at lower shear rates. A similar trend is observed when the CMC concentration is increased [35]. Using the cross model, $\lambda = 0.010$ s was reported for 0.2% CMC solutions (700,000 g/mol). These differences might be due to the molecular mass of the CMC samples and the method used to obtain the polysaccharide [35].

The parameter $p = (1-n)/2$, which is related to the flow behavior index n of the Oswald-de Waele model [15], varied slightly from 0.18 to 0.20 for the samples with fructans, except for CHF 6%; however, this parameter was close to that obtained in the CMC solution, which implies a similar viscosity dependence of the shear rate.

3.6 Viscoelastic properties of chickpea concentrate/carboxymethylcellulose aqueous mixtures

 Figure 2 shows *G*' and *G*'' as a function of the angular frequency (*ω*) of the CPC/CMC/fructan aqueous mixtures compared to those of the CMC solution. No predominant elastic properties $(G' > G'')$ were observed, since the selected concentration was far from the second critical concentration of CMC (c^{**}) , which reflects the transition to concentrated solutions (\geq 2.5%) [35].

Table 2. Carreau parameters of the CMC solution and aqueous CPC/fructan mixtures at 25 °C.

Sample	η_0 (Pa s)	$\lambda(s)$	
CMC	$0.076^{\circ} \pm 0.002$	$0.043^b \pm 0.003$	$0.211^{\circ} \pm 0.002$
CHF6-CMC	$0.146^a \pm 0.006$	$0.052^a \pm 0.004$	$0.242^a \pm 0.001$
CPC-CMC	$0.060^d \pm 0.002$	$0.026^{\circ} \pm 0.001$	$0.186^d \pm 0.002$
AGF3-CPC-CMC	$0.062^d \pm 0.000$	$0.026^{\circ} \pm 0.001$	$0.186^d \pm 0.003$
AGF6-CPC-CMC	$0.070^{\circ} \pm 0.000$	$0.030^{\text{de}} \pm 0.001$	$0.184^d \pm 0.001$
CHF3-CPC-CMC	$0.073^{\circ} \pm 0.003$	$0.035^{\text{cd}} \pm 0.001$	$0.186^d \pm 0.002$
CHF6-CPC-CMC	$0.101^b \pm 0.004$	$0.038^{bc} \pm 0.002$	$0.198^{\circ} \pm 0.005$

Carboxymethyl cellulose (CMC, 0.2%), other nomenclature as in Table 1. Tukey comparison by column, 95% confidence interval. Means that do not share a letter are significantly different.

Figure 2. Storage modulus (*G*') and loss modulus (*G*'') as a function of the angular frequency (ω) of the CMC solution and aqueous mixtures at 25 °C. Nomenclature as in Table 1 and Table 2.

 The mechanical spectrum of the CMC solution was similar to that of diluted or concentrated solutions, with a strong dependence on *ω* and *G*'' which was greater than *G*', implying a liquid-like behavior, up to a certain *ω* at point where this behavior could be inverted. When the moduli were adjusted to their respective power functions, Eq. 4 and Eq. 5, *α*'' was greater than *α*', which confirmed a predominantly liquid behavior (Table 3); the addition of CPC to the CMC solution (0.2%) decreased *α*'' by 50%, although the concentration of the CPC was 4%. This behavior was similar to the decrease observed in the viscosity curves as a function of shear rate.

 The opposite behavior was observed when 6% CHF was added to a CMC solution. *α*'' increased 114% and 86%, respectively, when 6% CHF was added to CPC/ CMC aqueous mixtures, in the same way as observed for the increase in overall viscosity. The exponents of the power functions of both moduli were close to unity, indicating a similar dependence on *ω*. The exponents for the CMC solution, $\beta' = 1.2$ and $\beta'' = 0.84$, increased to 1.41 and 0.92, respectively, when CPC was added, indicating a dependence on a more fluid material. The mixtures with 6% CHF had β ["] = 0.78-0.81, similar to the CMC exponent (Table 3).

3.7 Flow properties of emulsions

 As expected, the overall viscosity of the emulsions containing CPC/CMC aqueous mixtures and 30% oil was higher than the viscosity of the aqueous samples, and the shear-thinning curves are similar to those shown in Figure 1. This increase is due to the presence of dispersed oil droplets. Emulsions containing 30% oil showed viscosity shear-thinning curves characteristic of the CMC solution (Carreau model): constant viscosity at low shear rates (plateau) and shear-thinning behavior at high shear rates. Additionally, a thixotropic behavior was observed as the viscosity curves as a function of shear rate, obtained by successively increasing (upward curve) and decreasing (downward curve) the shear rates, did not coincide, and a hysteresis loop was detected (Figure S1). Thixotropy was also reported in sonicated emulsions (18% v/v oil) containing mixtures of galactomannans with weak gel structures [17]. In emulsions containing CPC/CMC aqueous mixtures, the CPC surrounded the dispersed oil droplets, and the CMC remained in the aqueous solution. In this case, thixotropy could be due to the formation of high-CMC-concentration domains within the emulsion (weak gel) and the breaking of the hydrogen bonds formed by CMC molecules.

 For comparative purposes and to confirm the effect of US on the properties of the CPC, the viscosity was measured without applying US and was included in the same figure (Figure S1). The overall viscosity of the emulsions decreased when the CPC was not subjected to US, confirming the improvement in the surface properties of the proteins, even in the 60% oil emulsions. In all cases, CMC limited the aggregation of the oil globules due to the increase in viscosity and the steric repulsion forces caused by the many carboxyl groups in its structure.

 Figure S2 displays the viscosity as a function of shear rate of the emulsions with 30% oil and the addition of fructans at two concentrations, 3% and 6%. All figures show the viscosity of the emulsions with the CPC/CMC aqueous mixture for comparison. As with the aqueous samples with CPC/CMC (Figure 1), it can be seen that an increase in the fructan concentration increases the overall viscosity, with emulsions containing 6% CHF having the highest viscosity.

 Table 4 shows that the Carreau parameters of the tested emulsions (30% oil) and η_0 (upward curve) increased with increasing fructan concentration, and the samples with CHF had significantly different η_0 values ($p < 0.05$) compared to those of the CPC/CMC emulsion. The η_0 corresponding to the downward curve also increased as the fructan concentration increased, with the η_0 values of AGF (6%) and CHF (6%) being significantly different (p < 0.05).

 The degree of thixotropy of the emulsion without fructans, measured as the difference in η_0 between the upward and downward curves, compared to the η_0 of the upward curve, expressed as a percentage, was 24%. The effect of fructans on thixotropy is unclear, even if the values are close, as the degrees of thixotropy were 27.6% and 22.1% for the AGF emulsions and 14.3% and 26.4% for the CHF emulsions at concentrations of 3% and 6%, respectively.

 The characteristic time, *λ* varied from 0.029 to 0.048 s (upward curve) and from 0.035 to 0.074 s (downward curve). λ was greater than that of the respective aqueous samples, indicating that the onset was slightly shifted to lower shear rates. A higher λ is related to a more complex structure, due to a higher concentration of macromolecules or the presence of several components. In the downward curve, *p* was lower than that of the respective aqueous samples, indicating that the shear-thinning dependence of the shear rate was lower.

 The viscosity dependence of the shear rate of emulsions containing 60% oil differed from that of the aqueous phase (Carreau model) and fitted the Herschel-Bulkley model (Figure 3). Thixotropic behavior was also observed in the emulsions studied. The rheological parameters were determined from the shear stress curve as a function of the shear rate and from the upward–downward curves. The consistency indices obtained for the downward curves were lower than those obtained for the upward curves, confirming thixotropic behavior (Table 5). As expected, the consistency index increases with increasing fructan concentration, particularly with CHF concentration. The flow behavior indices ranged between 0.45 and 0.53, indicating a strong dependence on the shear rate, with no tendency due to the addition of fructans. The yield stress significantly increased with the addition of fructans in proportion to the increase of the CHF concentration.

 The degree of thixotropy could be calculated by the decrease in the consistency index or the decrease in yield stress, as was done by η_0 . The values in the 60% oil emulsions were greater than those in the 30% oil emulsions. For example, for the consistency index, 37.2% of the emulsion contained only CPC/CMC, which increased to 53.7% if the protein was not sonicated. The highest yield stress values were observed for the samples with fructans, ranging from 48.5% to 89.6%, except for the sample containing 3% AGF (10.2%). The higher degree of

thixotropy resulted from the formation of a more complex structure, such as a three-dimensional network (weak gel and flocculated oil droplets), that was broken by the applied shear due to the increase in shear rate. The formation of gel-like structures in depleted emulsions by polysaccharide presence had been reported, in which a transient gel arose from the attractive depletion interactions between the oil droplets induced by the nonadsorbing polysaccharide [40].

Table 3. Parameters related to the storage modulus (*G*') and loss modulus (*G*'') with respect to the angular frequency of the studied samples, $G' = \alpha' \omega^{\beta'}$, $G'' = \alpha'' \omega^{\beta''}$ at 25 °C.

Sample	α' (Pa $s^{\beta'}$)		α'' (Pa s^p)	
CMC.	$0.018^{\circ} \pm 0.001$	$1.19^{\circ} \pm 0.024$	$0.136^{\circ} \pm 0.000$	$0.844^{\circ} \pm 0.007$
CPC-CMC	$0.003^{\circ} \pm 0.001$	$1.41^{\circ} \pm 0.004$	$0.068^d \pm 0.002$	$0.912^{\circ} \pm 0.008$
CHF6-CMC	$0.090^a \pm 0.004$	$0.967^{\circ} \pm 0.037$	$0.289^{\circ} \pm 0.009$	$0.780^4 \pm 0.008$
CHF6-CPC-CMC	$0.092^{\circ} \pm 0.003$	$0.876^{\circ} \pm 0.0037$	$0.251^b \pm 0.007$	$0.808^{\circ} \pm 0.005$

Nomenclature as in Table 1 and Table 2. Tukey comparison by column, 95% confidence interval. Means that do not share a letter are significantly different.

Nomenclature as in Table 1 and Table 2. Tukey comparison by column, 95% confidence interval. Means that do not share a letter are significantly different.

Figure 3. Viscosity as a function of shear rate of the studied emulsions (60% oil, E60) containing aqueous mixtures, (a) 3% AGF/ CPC/CMC, (b) 3% CHF/CPC/CMC, (c) 6% AGF/CPC/CMC, (d) 6% CHF/CPC/CMC; u and d represent the upward and downward flow curves, respectively. Nomenclature as in Table 1 and Table 2.

Table 5. Herschel-Bulkley parameters of the studied emulsions (60% oil, E60) at 25 °C.

	Yield stress (Pa)		Consistency index $(Pa sn)$		Flow behavior index (-)	
Sample	upward	downward	upward	downward	upward	downward
E60 CPC*-CMC	1.07 ^f \pm 0.058	0.867 ^f ± 0.058	$3.35^{\circ} \pm 0.188$	$1.55^d \pm 0.132$	$0.553^a \pm 0.008$	$0.675^{\circ} \pm 0.014$
E60 CPC-CMC	$8.00^{\circ} \pm 0.500$	$1.07^{\circ} \pm 0.058$	$6.50^{\rm b} \pm 0.637$	$4.08^{\circ} \pm 0.575$	$0.472^{bc} \pm 0.011$	$0.555^b \pm 0.017$
E60 AGF3-CPC-CMC	$10.4^d \pm 0.479$	$2.45^{\circ} \pm 0.041$	$7.47^{\rm b} \pm 0.532$	$5.21^b \pm 0.442$	$0.479^b \pm 0.009$	$0.545^b \pm 0.012$
E60 AGF6-CPC-CMC	$14.8^b \pm 0.216$	$4.19^b \pm 0.075$	$9.36^a \pm 0.327$	$7.11^a \pm 0.480$	$0.457^{\circ} \pm 0.004$	$0.509^{\circ} \pm 0.008$
E60 CHF3-CPC-CMC	$12.8^{\circ} \pm 0.058$	$1.33^d \pm 0.029$	$6.88^b \pm 0.641$	$5.74^b \pm 0.374$	$0.48^b \pm 0.014$	$0.517^{\circ} \pm 0.007$
E60 CHF6-CPC-CMC	$20.2^{\circ} \pm 0.764$	$10.4^a \pm 0.100$	$10.2^{\circ} \pm 0.187$	$7.52^a \pm 0.130$	$0.46^{bc} \pm 0.003$	$0.512^{\circ} \pm 0.002$

*Nonsonicated proteins. Nomenclature as in Table 1 and Table 2. Tukey comparison by column, 95% confidence interval. Means that do not share a letter are significantly different.

Figure 4. Storage modulus (*G*') and loss modulus (*G*'') as a function of angular frequency (*ω*) for the studied emulsions (30% oil, E30; 60% oil, E60) containing aqueous mixtures, (a) 6% AGF/CPC/CMC, (b) 6% CHF/CPC/CMC. Nomenclature as in Table 1 and Table 2.

3.8 Viscoelastic properties of the emulsions

 The viscoelastic properties of the emulsions with 30% or 60% oil were evaluated via oscillatory shear tests, and these oil concentrations exhibited different viscoelastic behaviors in the frequency sweeps performed in the smallamplitude oscillatory shear regime (Figure 4). Emulsions with 60% oil showed predominantly elastic behavior $(G' > G'')$ with a moderate frequency dependence in the range studied, which corresponded to a gel-like behavior where the molecular rearrangements within the oil droplet network (flocculated droplets) decreased over the time scales analyzed. In contrast, emulsions with 30% oil exhibited a high-frequency dependence, suggesting that relaxation processes occurred even at short time scales and that the difference between the moduli was lower, revealing a weaker structure caused by the concentrated CMC domains [37].

 For a periodic strain, the energy storage per cycle depended on *G*' and was contributed by the oil droplets as well as the polysaccharide molecules; the energy dissipation depended on *G*'' and had contributions from solute and solvent. The relative contributions of the solute to *G*' and *G*'' depended on the extent to which the Brownian motions were correlated with the external forces [38].

As shown in Figure 4, all the emulsions with 60% oil

exhibited a similar rheological behavior since the storage modulus was always greater than the loss modulus in the frequency range studied. A minimum in *G*'' and a lowfrequency dependence of *G*' were observed in all the cases. This behavior might be related to structural network formation in oil-in-water emulsions, possibly because of extensive flocculation processes that were confirmed by the detection of yield stress by flow tests.

 As shown in Table 6, the values of the constant *β*' are smaller than those of β " (from Eq. 5 and Eq. 6), which implies that the *G*' module was less dependent on the frequency than *G*''; *α*' values are also higher than *α*'' by an order of magnitude. The emulsions with fructans were more significantly elastic ($p < 0.05$), and CHF promoted a greater *α*'. This result was observed because if depletion caused flocculation, then a concentration of polysaccharide molecules existed in certain regions, which in turn provided a greater contribution to the elastic modulus due to more restricted movement between particles. For emulsions with 30% oil, *G*' and *G*'' evidently depended on the angular frequency. *G*' and *G*'' increased with increasing frequency, which could be interpreted as the network formed being composed mainly of weak bonds formed by concentrated CMC domains [39].

Table 6. Parameters related to the storage modulus (*G*') and loss modulus (*G'*') with respect to the angular frequency, $G' = \alpha' \omega^{\beta}$ and $G'' = \alpha'' \omega^{\beta'}$, of the studied emulsions at 25 °C.

Sample	α' (Pa s ^{β'})		α " (Pa s ^{β})	R ''
E30 CPC-CMC	$3.16^{\circ} \pm 0.226$	$0.453^{\circ} \pm 0.028$	$2.98^{\circ} \pm 0.220$	$0.465^{\circ} \pm 0.010$
E30 AGF6-CPC-CMC	$2.36^{\circ} \pm 0.230$	$0.423^{\circ} \pm 0.034$	$1.96^{\rm b} \pm 0.099$	$0.494^{\circ} \pm 0.003$
E30 CHF6-CPC-CMC	$6.09^{\circ} \pm 0.194$	$0.308^b \pm 0.012$	$3.00^{\circ} \pm 0.210$	$0.457^b \pm 0.007$
E60 CPC-CMC	$188.8^{\circ} \pm 7.8$	$0.088^a \pm 0.002$	$24.1^{\circ} \pm 1.25$	$0.24^{\circ} \pm 0.005$
E60 AGF6-CPC-CMC	$248.0^{\circ} \pm 5.8$	$0.082^b \pm 0.002$	$30.5^{\circ} \pm 1.10$	$0.22^b \pm 0.006$
E60 CHF6-CPC-CMC	$354.4^{\circ} \pm 10.6$	$0.084^{\circ} \pm 0.002$	$43.9^{\circ} \pm 1.54$	$0.20^{\circ} \pm 0.009$

Nomenclature as in Table 1 and Table 2. Tukey comparison by group and column, 95% confidence intervals. Means that do not share a letter are significantly different.

The α ' value was significantly lower ($p < 0.05$) for the CPC/CMC emulsions without fructans, illustrating that fructans affected the rheological properties of the emulsions. However, this effect was more evident in the case of emulsions with 60% oil. As a result, the values of *α*' and α ["] decreased significantly ($p < 0.05$) in emulsions with 30% oil with AGF in comparison even with the emulsion without fructans. Consequently, it could be considered that the aqueous phase made the main contribution to the viscoelastic behavior of these emulsions. The *G*' curves at 30% oil for AGF and CHF tended to approach each other at high frequencies, probably because the behavior was dominated by short-range configuration changes, which were oblivious to the branch points of the CHF; at low frequencies, *G*' values were higher for emulsions with

CHF, which reflected the well-known effect of branching on intrinsic viscosity [38].

 An increase in the oil fraction of the studied emulsions implied that the continuous phase, in which the oily phase was added, still contained the same amount of fructans and CMC, but its concentration also increased. Consequently, the viscosity of the continuous phase was greater. Some polysaccharides in the continuous phase could cause flocculation of the particles. This depletion flocculation arose when polymer molecules were excluded from the small gap between neighboring oil droplets because of their small size compared to the droplet size. The concentration difference thus caused an osmotic pressure difference between the bulk and the gap. This pressure difference resulted in the solvent leaving the gap,

thus pulling the particles together [40]. Consequently, the increase in the oil fraction of the emulsions studied implied that in the continuous phase, the fructan and CMC concentrations also increased, so the viscosity of the continuous phase increased.

 In sonicated emulsions containing mixtures of 1.5% (w/v) galactomannans and 18% sunflower oil, a weak gel behavior was observed; however, no predominant solid or fluid behavior was observed, as in the emulsions containing 60% oil and 30% oil, respectively, since the viscoelastic properties were close, reporting values of tan $\delta = G''/G'$ close to one [17].

3.9 Particle size of emulsions

 The particle size distribution profiles for fresh CPC/ CMC/fructan emulsions (30% and 60%) for AGF and CHF at different fructan concentrations are shown in Figure 5. The CPC/CMC emulsion profile was included for comparative purposes. The 30% oil emulsions with 3% AGF and 6% CHF showed droplet size distributions similar to those of the CPC/CMC emulsion, but with a slightly reduced peak value characterizing the particle size and representing the statistical mode (Table 7). Emulsions with 6% AGF and 3% CHF displayed a more monomodal size distribution with a significant ($p < 0.05$) reduction in relative span and peak mode.

 As the oil concentration increased from 30% to 60%, the average droplet size increased, possibly due to the decreased availability of protein as an emulsifier to cover the oil droplet surface and increased recoalescence during homogenization. When the protein was not sonicated, the particles were obviously larger, but these sizes were not significantly different ($p < 0.05$) from those of the sample with the sonicated protein. In contrast, the emulsions with the highest concentration of fructans had significantly smaller particle sizes (Table 7). This result could be attributed to reduced recoalescence due to their flow behavior.

Figure 5. Particle size distribution of emulsions with (a) 30% (E30) and (b) 60% (E60) oil containing aqueous mixtures. Nomenclature as in Table 1 and Table 2.

Table 7. Size distribution and relative span of the studied emulsions (30% oil, E30, 60% oil, and E60).

Sample	Peak (μm)	Relative span
E30 CPC-CMC	$9.75^{\mathrm{a}} \pm 0.057$	$2.11^a \pm 0.013$
E30 AGF3-CPC-CMC	$8.74^{\circ} \pm 0.136$	$2.08^{ab} \pm 0.012$
E30 AGF6-CPC-CMC	$6.31^{d} \pm 0.057$	$1.88^{\circ} \pm 0.013$
E30 CHF3-CPC-CMC	$5.98^d \pm 0.047$	$1.84^{\circ} \pm 0.012$
E30 CHF6-CPC-CMC	$9.14^b \pm 0.125$	$2.01^b \pm 0.061$
E60 CPC*-CMC	$11.81^{\text{d}} \pm 0.172$	$1.61^{bc} \pm 0.010$
E60 CPC-CMC	$13.88^{ab} \pm 0.241$	$1.59^{\circ} \pm 0.029$
E60 AGF3-CPC-CMC	$13.36^{bc} \pm 0.148$	$1.69^a \pm 0.009$
E60 AGF6-CPC-CMC	$13.15^{\circ} \pm 0.345$	$1.61^b \pm 0.045$
E60 CHF3-CPC-CMC	$14.25^{\circ} \pm 0.163$	$1.55^{\circ} \pm 0.003$
E60 CHF6-CPC-CMC	$11.99^d \pm 0.173$	$1.53^{\circ} \pm 0.017$

*nonsonicated proteins. Nomenclature as in Table 1 and Table 2. Tukey comparison by group (30% or 60% oil), and column, 95% confidence interval. Means that do not share a letter are significantly different.

Figure 6. Backscattering profiles of emulsions (30%, E30) containing the studied aqueous mixtures. Raw mode (a, c, d) and reference mode (b). Freshly made emulsions (0 d) and 7-day-old emulsions. Nomenclature as in Table 1 and Table 2

3.10 Stability of emulsions

 The BS profiles of the emulsions (30%) made with aqueous mixtures of CPC/CMC and AGF or CHF are shown in Figure 6 for freshly made emulsions (0 days) and 7-day-old emulsions. The profiles were constant over the entire height of the analyzed sample (Figure 6a, 6c, 6d) since the BS signal was greater than 80% and the T signal was zero. The BS profile of emulsions containing CPC/CMC aqueous mixtures is shown in Figure 6b. In this reference mode, the $\triangle BS$ profiles at t = 0 min were used as a baseline and subtracted from the profiles at other time points.

 According to changes in the ∆BS profile and the principle of measurement, we found that the emulsion containing CPC/CMC aqueous mixtures underwent destabilization after 7 days, which could be induced by the creaming of the dispersed droplets, which was due to a decrease in the amount of BS at the bottom of the emulsions (clarification zone) over time because of the upward motion of the droplets by gravity (Figure 6b). A classic creaming profile was described for diluted emulsions (3.2% oil) with 3.5% CPC in the aqueous phase, in which three zones can be distinguished: a lower layer, with few or no droplets; an intermediate layer, with approximately the same droplet concentration as the original emulsion; and an upper layer, in which the droplets are tightly packed [14].

 The addition of AGF or CHF to aqueous systems containing CPC/CMC led to the formation of kinetically stable emulsions during the studied period, since the BS profiles of the emulsions (60% oil) were constant over the entire height of the analyzed sample (Figure 6a, 6b, 6c). In the presence of AGF or CHF, the internal droplet diameter decreased, probably because of the formation of a denser layer at the interface generated by the possible electrostatic interactions between the protein and the fructans, resulting in an efficient stabilization of the emulsions. Therefore, the long-term stability of the emulsion is related to the particle size distribution of the internal phase and the rheological properties of the emulsion [13, 14].

Conclusion

 Emulsions containing 30% oil showed shear-thinning viscosity curves characteristic of CMC (Carreau model) and thixotropic behavior. Despite the liquid-like behavior of the aqueous samples containing AGF (6%), the CPC/CMC characteristic of concentrated solutions was observed. The mechanical spectra exhibited few differences in modulus values and a high dependence on *ω*, suggesting that the concentrated CMC domains weakened the structure.

 Emulsions with 60% oil showed yield stress and shearthinning behavior characteristic of the Herschel-Bulkley model and thixotropic behavior. The mechanical spectra showed a predominantly elastic behavior with a moderate dependence on *ω*, which corresponds to a gel-like structure of a flocculated droplet network. The addition of AGF or CHF promoted an increase in overall viscosity and reduced the globule size of emulsions containing CPC/CMC aqueous mixtures, which improved the stability of the emulsion.

 Importantly, the CMC (0.2%) rheology defined by the Carreau model predominates in all aqueous systems, and the synergistic effect of 6% CHF on the CMC viscosity was attributed to the formation of interpolymer complexes between both biopolymers. In contrast, CPC interfered with the 0.2% CMC solution and decreased the overall viscosity (21%), but this did not affect the emulsion characteristics of the three polymers.

 This research confirms the usefulness of incorporating commercial CPC and fructans into healthy plantbased products, such as CMC-stabilized oil-in-water emulsions that can be used as delivery systems for other nutraceuticals, in light or concentrated dressing-type products such as mayonnaise.

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Authors' contributions

 LPMP planned and designed the study, analyzed the data, wrote and edited the manuscript. MGSH, planned and designed the study, performed measurements, analyzed the data, wrote and edited part of the manuscript. MRP, prepared the samples, performed measurements, wrote up some methods and results, analyzed the data. BJM, developed part of the experiments and data analysis. All authors have read and approved the final version of this manuscript.

Conflict of interests

 The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the research reported in this paper.

Supplementary materials

 The supplementary materials of this research (Figure 1S, Figure 2S) are available at [https://file.luminescience.](https://file.luminescience.cn/FNDS-249%20Supplementary%20Figures.pdf) [cn/FNDS-249%20Supplementary%20Figures.pdf.](https://file.luminescience.cn/FNDS-249%20Supplementary%20Figures.pdf)

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