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Rheological and Stability Evaluation of Emulsions Containing Fenugreek Galactomannan—Xanthan Gum Mixtures: Effect of Microwave and Ultrasound Treatments

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Abstract: The effects of treating two biopolymers (*Trigonella foenum—graceum* galactomannan and xanthan gum mixtures) with microwaves and ultrasound on the rheological aspects of O/W emulsions were investigated. The data obtained from steady shear flow were fitted with various models and the best were chosen due to the values of R^2 and RMSE. The oscillatory shear rheology data demonstrated that the emulsions not treated with microwaves or ultrasound had viscous-like behavior and treated samples demonstrated weak gel behavior. The values obtained for various rheological parameters (especially apparent viscosity, storage modulus and loss modulus) indicated that fenugreek galactomannan had more impact on the rheological aspects of emulsions in comparison with xanthan gum. In addition, the synergistic interaction between two biopolymers, particularly in samples treated with ultrasound, resulted in better rheological aspects which could be affiliated with the strong bonds between the hydrocolloids. By treating the samples with microwaves and ultrasound, the emulsion stability values of the samples (especially those with a high ratio of galactomannan) significantly increased, which might be connected with various parameters, especially viscosity.

Keywords: ultrasound; microwave; emulsion; galactomannan; xanthan gum; rheological properties

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1. Introduction

Emulsions, as an unstable system, have a vital role in various fields especially the pharmaceutical, cosmetics and food industries [1]. The instability of these systems causes them to breakdown during storage which is directly associated with consumer dissatisfaction [2]. Emulsion breakdown is mainly affiliated with various mechanisms, especially flocculation and creaming. In most cases, stabilizers are utilized to improve the stability of emulsions during storage [3]. Emulsions generally demonstrate three main rheological behaviors consisting of Newtonian (where fluid flow remains constant with shear rate), shear-thinning, (where fluid flow diminishes with shear rate) and shear-thickening (where fluid flow elevates with shear rate). Any factor that changes the flow behavior can govern emulsion stability. Among the factors, utilizing hydrocolloids as stabilizers or emulsifiers, especially in binary systems, has become more acceptable in recent years [4].

Hydrocolloids are well-known compounds used as thickeners, stabilizers, emulsifiers and gels in various food formulations [5,6].

Fenugreek (*Trigonella foenum—graceum*) is a crucial plant with various known phytochemicals that is harvested in various regions, especially in the west part of Asia [7]. The galactomannan obtained from fenugreek seed has high carbohydrate and low protein and fat content [8,9]. Former research has demonstrated that fenugreek seed galactomannans have the ability to construct better and more stable emulsions than other galactomannans

including commercial galactomannans like guar gum or locust bean gum (LBG). Therefore, the production of O/W emulsions containing this hydrocolloid can be helpful for researchers in the food industry [10].

Xanthan gum, an extracellular bacterial biopolymer from *Xanthomonas campestris*, is a vital industrial hydrocolloid. It constructs an acceptable rigid rod-like conformation in solution at ambient temperature; in addition, it converts to a random structure when heated. These rods have the ability to align themselves to form proper rigid mixed gels with various utilizations in the food industry. An important advantage of this biopolymer is its stability in a broad range of pHs and temperatures. Previous studies have shown that xanthan gum has unique flow properties that are attributed to the structure mentioned above (rigid rod-like conformation) [11].

Microwave treatment is a simple, relatively safe and common process with numerous advantages including precise process control and being cost effective. This treatment can remarkably affect numerous aspects of emulsions. Scrutinizing the effect of this treatment on the rheological aspects of emulsions (especially when containing hydrocolloids) as a main part of physicochemical properties, could be considered as novel for researchers [12,13].

Xanthan gum is mostly blended with galactomannans like guar gum and LBG in binary gum mixture structures that are attributed to their synergism of viscosity as a result of intermolecular interaction between xanthan gum and mannan molecules [14,15]. The functional aspects of hydrocolloids are affiliated with the molecular parameters of each biopolymer and the nature of interactions between them [6]. Kennedy et al., 2015, Koop et al., 2009 and Tako et al., 2010 worked on the interaction of mixed xanthan gum—LBG, xanthan gum—galactomannan obtained from the seeds of *Mimosa scabrella*, and xanthan gum—*Delonix regia* seed galactomannan utilizing the steady shear flow and dynamic rheological aspects [16–18]. They found that the flow curves of mixed solutions at low concentration demonstrated plastic behavior, showing synergistic interaction between xanthan and galactomannan molecules [18,19].

In order to provide proper data on the physical stability of the emulsions, measuring rheological aspects is very important [1]. Hydrocolloids, especially galactomannans as rheology modifiers, can govern the consistency of emulsions and can adsorb on interfaces at multiple sites. The galactomannan source and its mannose to galactose ratio can affect the emulsion stability and capacity [20]. Data obtained from former studies authenticated that the availability of two hydrocolloids in the formulation of emulsions can enhance their stability. Qiu et al. 2015 studied the effect of xanthan gum and pectin on the stability of emulsions. The obtained results demonstrated that by adding these two hydrocolloids the stability of emulsions was improved, which was in line with the rheological data [21,22]. Also, the effect of xanthan gum and guar gum on the rheological aspects of O/W emulsions was studied and the results confirmed the positive effect of using two hydrocolloids on the rheological aspects of emulsions [13].

Hence, the main aim of the current work was to investigate the effect of adding two known hydrocolloids (fenugreek galactomannan and xanthan gum) treated with microwaves and ultrasound on the rheological aspects of emulsion samples.

2. Material and Methods

2.1. Raw Material and Chemical

Fenugreek seeds were acquired from Tabriz, Iran. Xanthan gum powder extracted from *Xanthamonas campestris* was obtained from the Department of Microbiology, Al-Zahra University, Iran.

2.2. Fenugreek Galactomannan Extraction

To accomplish this procedure, 100 g of the *Trigonella foenum—graceum* (fenugreek) seeds were completely cleaned and grounded. To achieve high extraction efficiency, proper hydration of the seeds, and to eliminate the possible existing impurities, seeds which were completely powdered were put in a water bath at a high temperature (at about 80 °C) for

about 15 min. Then, they were thoroughly mixed with deionized water (1:10) and put on a stirrer for 1 h at 45 °C. Subsequently, to separate the mucilage from the seeds, an extractor was applied. Extracted mucilage was blended with ethanol (96%) to precipitate galactomannan. Two crucial stages were done to completely purify the galactomannan. In the initial stage, the precipitated galactomannan was mixed with deionized water and the obtained solution was centrifuged. In the second stage, the solution was washed several times with ethanol (96%) and acetone to omit different varieties of impurities, particularly those that could have adverse effects on the experiments. After that, the filtered hydrocolloid was put in an oven (45 °C for 12 h) to accomplish the drying procedure. At last, the dried galactomannan was completely powdered and put in proper storage until its application in the film formulation [23].

2.3. Xanthan Gum Extraction

A loopful of bacterial cells was transferred from an overnight culture on a yeast malt (YM) agar slant to a test tube containing 5 mL of YM broth medium and was incubated in a shaker incubator at 28 °C for about 8 h. The absorption was then measured at 600 nm, as about 0.5, and inoculated at 2% (v/v) into the same preculture medium in a 100-mL flask. The preculture flask containing 20 mL of YM broth was incubated at 28 °C and 140 rpm for a further 8 h and transferred to the production medium in a baffled flask containing a nutrient salt solution with 2% glucose and incubated for 3 days resulting in a highly viscous solution. To extract the biopolymer, after stirring the fermentation liquid, a NaCl solution (final concentration 0.3% w/v) was added per 100 mL of fermentation liquid and then completely mixed. Subsequently, while stirring, 1.7 to 2 times the volume of the fermentation liquid isopropanol was added. The resulting precipitate was dissolved in 1% KCl solution and vigorously shaken, and precipitation procedures were repeated to separate the cells and pigments from the biopolymer. Finally, the biopolymer precipitate was dried at 50 $\pm$ 5 °C for 24 h in an oven. The crude product (1% w/v) was dissolved in KCl (1% w/v) and heated for 30 min at 65 °C. After that stage, the solution pH was adjusted to 7.8 by utilizing sodium hydroxide and the dissolved cellulolytic enzyme cocktail was added to it and stirred for about 10 h at 75 °C. The cell mass was removed from the precipitated solution to purify the biopolymer [24].

2.4. Preparation of O/W Emulsions

The aqueous phase of samples was constructed by dispersing two biopolymers (fenugreek galactomannan and xanthan gum) with 1.5% w/v concentration in deionized water (100 cc) at 68 °C for 2 h. Obtained solutions were processed at a microwave power of 500 W in a microwave oven (Boutan, Iran). Sunflower oil was added calmly to produce the oil phase of samples. It must be mentioned that sunflower oil is a non-volatile oil pressed from the seeds of the sunflower and is composed of linoleic acid and oleic acid, which are well-known unsaturated fatty acids. Then, the emulsions were homogenized (at 20,000 rpm) (Ultra Turrax, IKA—WERKE, Staufen, Germany). In samples treated with ultrasound, the homogenization was done by ultrasound with an amplitude of 50% [2]. To better and more comprehensively investigate the effects of both biopolymers (fenugreek galactomannan (FG) and xanthan gum (XG)) treated with microwaves and ultrasound, 5 samples treated with microwaves and 5 treated with ultrasound (100FG, 100XG, 50FG50XG, 75FG25XG and 25FG75XG) were produced and also, control samples (without treatment) were constructed. The naming of these samples was based on the English alphabet, as shown in Table 1. Furthermore, 100, 50, 75, and 25 are the proportions of each utilized biopolymer.

Table 1. The list of emulsions containing two biopolymers treated with microwaves and ultrasound.

Sample Name	Sample Type	Hydrocolloids
A	Control	100FG
B	Control	100XG
C	Control	50FG50XG
D	Control	75FG25XG
E	Control	25FG75XG
F	Microwave treated	100FG
G	Microwave treated	100XG
H	Microwave treated	50FG50XG
I	Microwave treated	75FG25XG
J	Microwave treated	25FG75XG
K	Ultrasound treated	100FG
L	Ultrasound treated	100XG
M	Ultrasound treated	50FG50XG
N	Ultrasound treated	75FG25XG
O	Ultrasound treated	25FG75XG

2.5. Rheological Measurements

A Physica MCR301 rheometer (Anton Paar, GmbH, Graz, Austria) (25 °C) with a sample volume, diameter parallel plate geometry and gap of 20 mL, 50 mm and 0.206 mm was applied to scrutinize rheological features.

2.5.1. Steady Shear Flow Rheology

To evaluate shear stress—shear rate and apparent viscosity—shear rate data of the samples, a shear rate range of 0.1–100 s^{-1} was utilized. Shear stress (σ)—shear rate ($\gamma\cdot$) data were fitted with power law and Herschel—Bulkley and the second type of data was fitted with Carraeu models:

$$\sigma = k(\gamma\cdot)^{n_p} \quad (1)$$

$$\sigma = k(\gamma\cdot)^{n_p} \quad (2)$$

$$\eta_a = \eta_\infty + \frac{(\eta_0 - \eta_\infty)}{[1 + (\gamma\cdot/\gamma\cdot_c)^2]^{m\cdot}} \quad (3)$$

where k, σ_0, n, η_∞, $\gamma\cdot_c$ and m are consistency coefficient ($Pa.s^n$), yield stress (Pa), flow behaviour index, zero-shear viscosity (Pa.s), infinite-shear viscosity (Pa.s), critical shear rate (s^{-1}) and dimensionless exponent [25–29].

2.5.2. Dynamic Oscillatory Rheology

Strain Sweep

These computations (shear rate range of 0.1–10% and constant frequency of 1 Hz) were applied to scrutinize the linear viscoelastic region (LVR) of emulsions and estimation of factors, especially storage modulus, loss modulus and loss tangent [30].

Frequency Sweep

This computation was accomplished (angular frequency range of 0.1–100 Hz and constant shear rate of 0.1%) to investigate the viscoelastic aspects of emulsions, and various factors, especially complex viscosity (η*) as a function of frequency (ω), were scrutinized [29].

2.6. Emulsion Stability (ES)

Emulsions were put in glass containers and placed at room temperature for 28 days. The alterations were computed at the same day at each week. The ES was calculated by Equation (4):

$$ES = (\frac{e_v}{t_v}) \times 100 \quad (4)$$

where e_v is the emulsion volume and t_v is the total volume including the serum and cream phase.

2.7. Statistical Analysis

MATLAB (R2016a) was utilized to fit the mentioned models. Also, Rheoplus software (32 bit, version V3.40, Austria) was utilized to analyze the dynamic rheology data.

3. Results and Discussion

3.1. Steady Shear Flow Rheological Aspects

3.1.1. Fitting on Shear Stress—Shear Rate Based Models

The utilization of biopolymers in emulsions as a stabilizing agent is done by altering the value of continuous phase viscosity [2]. Figure 1 demonstrates the η_a of samples containing two hydrocolloids, most of which were treated with microwaves or ultrasound. The apparent viscosity values of the emulsions were elevated by treating them with microwaves and ultrasound. This elevation in viscosity is probably attributed with the construction of stronger bonds due to the interaction between the hydrocolloids, especially in the control samples [12,31]. The viscous nature of xanthan gum and fenugreek galactomannan resulted in an elevation in the emulsion continuous phase viscosity which resulted in better stabilizing properties [32]. Xanthan gum (XG) generally caused lower emulsion viscosity in comparison with fenugreek galactomannan (FG) at identical concentrations, which was also reported for guar gum [33]. As represented in Figure 1, the η_a of the samples were diminished with elevation of the shear rate that demonstrated their shear-thinning behavior. The η_a values of the samples were remarkably elevated by an elevation in FG concentration (Sample N had the highest viscosity). Also, the η_a value of emulsions containing XG alone was lower than other samples, especially when not treated with microwaves or ultrasound. Therefore, it can be inferred that by adding two hydrocolloids to the emulsion formulation and treating them with microwaves and ultrasound, which causes strong intramolecular interactions between them, the rheological properties of emulsions, especially flow properties, can be improved. It should also be noted that the type and amount of galactomannan added and also the ratio of galactomannan to other hydrocolloids used, in the values of the obtained parameters have a significant effect [11]. As demonstrated in Table 2, among the two models utilized for data fitting, the power law model provided more acceptable results than the other model. This suitability was confirmed by two parameters including R^2 and RMSE. The R^2 values of all emulsions in the power law model were between 0.97 and 0.99. In addition, the RMSE values were in the range of 0.00015 to 0.00326. The flow behavior index (n) value in the power law model is known as the indicator of shear-thinning behavior [22]. This parameter diminished (from 0.99 to 0.45) by treating samples with microwaves and especially, ultrasound. Existence of fenugreek galactomannan in the formulation of emulsions led to higher n values in comparison with xanthan gum. As a general rule, lower n values resulted in more noticeable shear-thinning behavior [11]. In addition, as demonstrated in Table 2, the values of n significantly decreased in emulsions treated with ultrasound. This higher shear-thinning behavior could result in the reduction of organoleptic sliminess during swallowing, which is associated with a pleasant and light mouthfeel [34]. In addition, the K and σ_0 values of the samples rose after being treated with microwaves and ultrasound. In emulsions treated with ultrasound, the higher values of yield stress demonstrated high stability potential against creaming in these samples. Contrarily, emulsions without any treatment represented weaker gel structure [35]. The same result was reported in samples containing guar gum—xanthan gum mixtures [11]

and xanthan gum—LBG mixtures [36] as well-known biopolymers. At last, it must be mentioned that samples N (75FG25XG) and B (100XG), had the highest and lowest ηa values. In addition, sample N had the highest k value and lowest n value between emulsions.

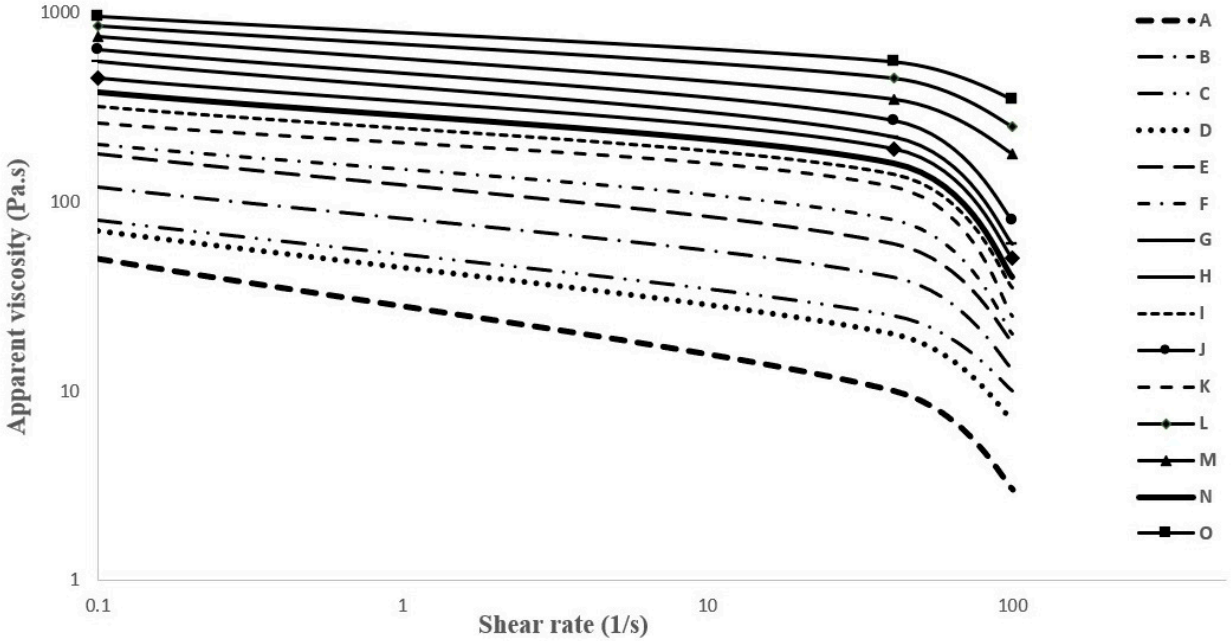

Figure 1. Apparent viscosity of the samples with two biopolymers (FG and XG) as a function of shear rate (0.01–100 s^{-1}).

Table 2. The results of fitting data of emulsions containing two hydrocolloids (fenugreek galactomannan and xanthan gum) with two known models.

Samples	Power Law Model				Herschel—Bulkley Model			
	K_p (Pa.s^n)	n_P	R^2	RMSE	σ_0 (Pa)	k_H (Pa.s^n)	R^2	RMSE
A	2.70 ± 0.06	0.98 ± 0.12	0.99	0.00015	1.98 ± 0.37	2.30 ± 0.12	0.91	0.00185
B	0.98 ±0.02	0.94 ± 0.08	0.98	0.00217	0.50 ± 0.29	0.72 ± 0.24	0.91	0.00415
C	2.34 ± 0.22	0.96 ± 0.21	0.98	0.00219	1.64 ± 0.74	2.11 ± 0.31	0.90	0.00562
D	2.98 ± 0.41	0.99 ± 0.47	0.99	0.00012	1.69 ± 0.39	2.25 ± 0.52	0.89	0.00985
E	1.66 ± 0.18	0.95 ± 0.19	0.97	0.00326	0.85 ± 0.17	1.31 ± 0.09	0.90	0.00511
F	4.60 ± 0.37	0.71 ± 0.05	0.99	0.00005	3.89 ± 0.18	4.12 ± 0.25	0.90	0.00489
G	1.96 ± 0.11	0.76 ± 0.08	0.98	0.00036	0.81 ± 0.24	1.26 ± 0.31	0.88	0.00891
H	3.78 ± 0.64	0.74 ± 0.15	0.99	0.00018	2.89 ± 0.16	3.32 ± 0.33	0.89	0.00745
I	4.69 ± 0.09	0.67 ± 0.05	0.99	0.00069	3.95 ± 0.14	4.27 ± 0.18	0.90	0.00488
J	2.98 ± 0.34	0.75 ± 0.05	0.98	0.00005	1.78 ± 0.34	2.37 ± 0.47	0.88	0.00811
K	6.45 ± 0.14	0.48 ± 0.02	0.99	0.00041	6.04 ± 0.17	6.18 ± 0.29	0.89	0.00852
L	3.92 ± 0.28	0.57 ± 0.05	0.99	0.00036	2.98 ± 0.11	3.41 ± 0.36	0.91	0.00325
M	5.96 ± 0.45	0.52 ± 0.08	0.98	0.00215	4.85 ± 0.24	5.55 ± 0.47	0.91	0.00258
N	6.61 ± 0.71	0.45 ± 0.12	0.99	0.00014	5.60 ± 0.14	6.26 ± 0.39	0.90	0.00333
O	4.59 ± 0.15	0.55 ± 0.16	0.97	0.00145	3.84 ± 0.11	4.18 ± 0.27	0.88	0.00774

3.1.2. Fitting on Apparent Viscosity—Shear Rate Based Model

The Carreau model, which is proper and suitable for illustration of shear-thinning behaviour, was utilized to fit the data (Table 3). This model demonstrated high R2

(0.9736–0.9936) and low RMSE (0.00147–0.08856). When treating the emulsions with microwaves and ultrasound, the zero shear viscosity (η0) value of the samples increased. As represented in Table 3, samples B and N had the lowest (75.60 Pa) and highest (951.45 Pa) zero shear viscosities. Fenugreek galactomannan had more impact on the viscosity in comparison with xanthan gum. Furthermore, as shown in Table 3, the emulsions treated with ultrasound had more zero shear viscosity values compared to emulsions containing one hydrocolloid (FG or XG). This might be affiliated with the synergistic interaction of the biopolymers related to the ultrasound treatment [15]. In most cases, due to the significant difficulty of estimating the η∞ value in food dispersions, this parameter is omitted in rheological calculations to avoid probable errors [2]. Usually, $\gamma \cdot^{c}$ in the model, offers an order of magnitude of the critical shear rate indicating the end of the Newtonian region or the initial point of the shear-thinning region. The value of the current parameter was reduced by treating the samples with microwaves and ultrasound. Sample B had the highest (4.4546 s^{-1}) and sample N (1.3563 s^{-1}) had the lowest value of critical shear rate among the samples. The parameter of m in the model is a dimensionless exponent that reduced when treating the samples, especially with ultrasound.

Table 3. The results of fitting data of emulsions containing two hydrocolloids (fenugreek galactomannan and xanthan gum) with the Carraeu model.

Samples	Carraeu Model		m		
	η_0 (Pa.s)	γ_c (s^{-1})		R^2	RMSE
A	212.30	3.3723	0.4725	0.9815	0.03756
B	75.60	4.4546	0.5325	0.9917	0.07496
C	150.31	3.6937	0.4817	0.9836	0.06347
D	250.25	3.1641	0.3919	0.9914	0.00364
E	98.36	4.2162	0.5117	0.9736	0.00147
F	587.50	2.3515	0.2547	0.9855	0.04866
G	103.26	3.9519	0.4950	0.9841	0.01833
H	351.78	2.8125	0.3476	0.9936	0.02256
I	695.36	1.9663	0.1826	0.9925	0.08856
J	303.25	3.0149	0.3715	0.9911	0.00963
K	887.96	1.7583	0.1537	0.9900	0.07458
L	400.21	2.6925	0.3125	0.9815	0.08633
M	795.38	1.3818	0.1154	0.9836	0.07745
N	951.45	1.3563	0.1037	0.9874	0.00963
O	491.74	2.4871	0.2811	0.9872	0.00854

It should be noted that according to the results obtained from fitting different models, fenugreek galactomannan, compared to xanthan gum, had a greater effect on the results, particularly viscosity, as a vital parameter. Additionally, the synergistic interaction of the two hydrocolloids (mostly due to the microwave and ultrasound treatment) had a significant effect on the results, which was a positive and improving effect.

3.2. Dynamic Rheological Aspects

3.2.1. Strain Sweep Test

LVR is known as an indicator of structure strength. A longer LVR demonstrates more stability and suitable strength of the sample [29]. Among the samples, emulsions treated with microwaves and especially ultrasound had a longer LVR. Among them, sample N (75FG25XG) had the longest LVR. As illustrated in Figure 2a,b, it was viable to scrutinize

two main sections in the current test, LVR and a non-linear one that G′ and G″ begin to diminish by elevating strain [2]. Various factors in the LVR were computed by this test; particularly, yield strain (γ_L) at the limit of the LVE region, G′, G″ and loss tangent (tanδ) values. The γ_L values of the samples were 0.147–2.168% (Table 4). G′ values of ultrasound-treated emulsions containing biopolymers (FG and XG) were higher than G″ values in the LVE region that illustrated elastic behavior in these samples. The elastic behavior is mostly affiliated with more intermolecular entanglements and indicates the strength of constructed network (related to the effect of treatment with microwaves and ultrasound) [11]. The ratio of G″ to G′ in each cycle is known as tanδ, which is a proper indicator for scrutinizing the viscoelastic behavior. A tanδ value lower than one demonstrates elastic behavior and values higher than one demonstrate viscous behavior. As shown in Table 4, the obtained values for the control samples (without being treated by microwaves or ultrasound) were higher than one and among them, sample B (100XG) had the lowest value (1.0891). On the other hand, in emulsions treated with microwaves and ultrasound, values were lower than one and among them, sample N (75FG25XG) had the lowest values (0.9189). Therefore, it can be concluded that the availability of FG in the formulation of emulsions (especially in samples treated with ultrasound) had more impact on the elastic behavior than XG. It must be mentioned that ultrasound and microwave treatments could play an important role in the flocculation of oil droplets which result in alterations in the rheological properties of emulsions. The elastic behavior is affiliated with the stabilizing strength in the O/W emulsions [21]. The G′ values of samples with a mixture of hydrocolloids (except those with more XG in the formulation of emulsion) were higher than samples with only one hydrocolloid that indicated the synergistic interaction of two biopolymers. This interaction resulted in a more acceptable elastic weak gel network [33]. It must be mentioned that this mechanism (interaction quality between xanthan gum and galactomannan) is mostly affiliated with the total available galactose and its distribution in the structure [11]. The same results were reported in emulsions containing xanthan gum—guar gum [32], xanthan gum—LBG [11], xanthan gum—galactomannan [18] and xanthan gum—CMC mixtures [17]. In all of the reported results, the storage modulus values of the samples with mixture hydrocolloids were higher than the values of samples with one hydrocolloid. In addition, in most of the samples, the G′ values were more than the values reported for G″.

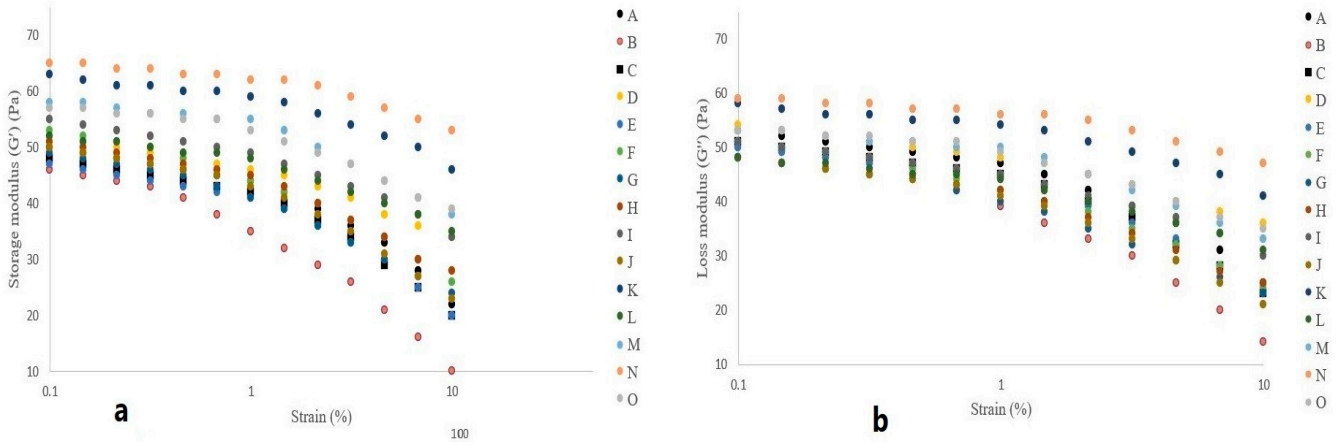

Figure 2. (**a**) Strain sweep profile of the samples with two biopolymers (FG and XG) for G′ changes (Angular frequency: 1 rad/s) (**b**) Strain sweep profile of the samples with two biopolymers (FG and XG) for G″ changes (Angular frequency: 1 rad/s).

Table 4. Strain sweep parameters of the emulsions containing two hydrocolloids (fenugreek galactomannan and xanthan gum) (strain% = 0.1–10%, angular frequency: 1 rad/s).

Samples	G′ (Pa) (Storage Modulus)	G″ (Pa) (Loss Modulus)	Tanδ	γ_L (Pa) (Yield Strain)	γ_f (Pa) (Flow Point Strain)
A	50.32 ± 0.37	53.74 ± 0.22	1.0679 ± 0.0025	0.216 ± 0.003	0.317 ± 0.003
B	46.24 ± 0.19	50.36 ± 0.25	1.0891 ± 0.0014	0.147 ± 0.015	0.216 ± 0.045
C	48.79 ± 0.25	51.49 ± 0.08	1.0553 ± 0.0085	0.317 ± 0.048	0.464 ± 0.345
D	52.36 ± 0.58	54.56 ± 0.79	1.0420 ± 0.0018	0.317 ± 0.002	0.464 ± 0.065
E	47.98 ± 0.45	50.18 ± 0.06	1.0558 ± 0.0076	0.216 ± 0.004	0.317 ± 0.008
F	53.69 ± 0.68	51.48 ± 0.18	0.9588 ± 0.0086	0.317 ± 0.065	0.464 ± 0.041
G	49.98 ± 0.28	48.85 ± 0.49	0.9773 ± 0.0049	0.216 ± 0.009	0.317 ± 0.031
H	51.18 ± 0.38	48.64 ± 0.17	0.9503 ± 0.0036	0.464 ± 0.014	0.682 ± 0.014
I	55.18 ± 0.24	51.94 ± 0.06	0.9412 ± 0.0019	0.464 ± 0.065	0.682 ± 0.025
J	50.22 ± 0.76	48.85 ± 0.18	0.9727 ± 0.0038	0.317 ± 0.005	0.464 ± 0.047
K	63.38 ± 0.74	58.84 ± 0.26	0.9288 ± 0.0014	0.682 ± 0.087	1.005 ± 0.052
L	52.87 ± 0.26	48.19 ± 0.49	0.9314 ± 0.0008	0.464 ± 0.007	0.682 ± 0.002
M	58.25 ± 0.38	53.98 ± 0.07	0.9266 ± 0.0004	1.005 ± 0.014	1.478 ± 0.032
N	65.14 ± 0.17	59.86 ± 0.76	0.9189 ± 0.0015	1.478 ± 0.068	2.160 ± 0.074
O	57.65 ± 0.22	53.25 ± 0.04	0.9286 ± 0.0087	1.001 ± 0.008	1.478 ± 0.024

3.2.2. Frequency Sweep Test

The obtained data are illustrated in Figure 3a,b and Table 5. G′ values of the control samples containing hydrocolloids were lower than G″ values at the utilized frequency. Therefore, these samples indicated the behavior of dilute solutions. Among these emulsions, sample B indicated the lowest G′ (30.25 Pa) and G″ (40.37 Pa) values and sample D demonstrated the highest G′ (42.25 Pa) and G″ (44.25 Pa) values. Conversely, the G′ values of emulsions treated with microwaves and ultrasound were higher than the G″ values that represented the weak gel behavior (attributed to the construction of bonds with high strength). Among these samples, sample G represented the lowest G′ (66.85 Pa) and G″ (58.26) values and sample N represented the highest G′ (93.58 Pa) and G″ (76.11 Pa) values. The obtained results were in line with the strain sweep data. The value of tanδ can additionally have a vital role in better determining the behavior of samples. Values of this parameter in control emulsions were higher than 1 (1.0473 and 1.3345 for samples D and B as the lowest and highest values) which indicated the viscous-like behaviour and in treated samples, these values were lower than one (0.8133 and 0.8715 for samples N and G as the lowest and highest values) which demonstrated the gel-like behaviour. By elevating the frequency, η* values of the samples diminished. The η* of the emulsions increased by treating them with microwaves and ultrasound. The highest and lowest values were obtained from sample N (75FG25XG) (30.14 Pa.s) and sample B (100XG) (12.11 Pa.s). The slope of η* versus frequency is known to be important in determining gel strength. A higher value of slope indicates the availability of proper and acceptable interactions in weak gel conformation that might be affiliated with various factors, especially the concentration of stabilizer in dispersion and treatment with microwaves or ultrasound [2,11]. The η*—frequency slope near to −0.80 demonstrates the weak gel characteristics of a sample [9]. The slope values of ultrasound-treated emulsions were significantly near to −0.8 and the nearest value was obtained by sample N (0.79). In addition, the slope values for control and microwave-treated samples were lower than emulsions treated with ultrasound. It must be mentioned that the synergistic interaction between two hydrocolloids (FG and XG), especially in samples treated with ultrasound, resulted in higher G′ and η* values, which

indicated strong bonds between the molecules of biopolymers. Additionally, fenugreek galactomannan had more impact on various rheological parameters investigated in the frequency sweep in comparison with xanthan gum. This was in agreement with the data obtained in former sections. An identical result was reported in emulsions containing guar gum—xanthan gum [11], xanthan gum—cress seed gum [6], xanthan gum—LBG [31] and xanthan gum—glucomannan [34] mixtures.

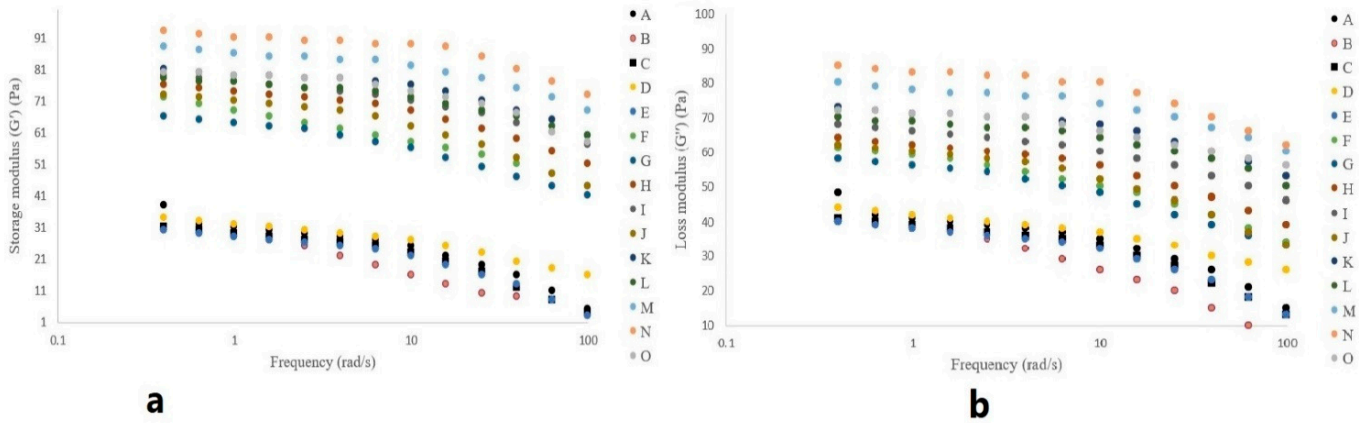

Figure 3. (**a**) Frequency sweep profile of the samples with two biopolymers (FG and XG for elastic modulus (G′) changes (Strain: 0.1%) (**b**) Frequency sweep profile of the samples with two biopolymers (FG and XG) for loss modulus (G″) changes (Strain: 0.1%).

Table 5. Frequency sweep parameters of the emulsions containing two hydrocolloids (fenugreek galactomannan and xanthan gum) (Angular frequency = 0.1–100%, strain%: 0.1%, 25 °C).

Samples	G′ (Pa)	G″ (Pa)	Tanδ	η* (Pa.s)	Slope of η*—f
A	38.54 ± 0.22	42.85 ± 0.10	1.1118 ± 0.0025	15.37 ± 0.12	−0.61 ± 0.03
B	30.25 ± 0.14	40.37 ± 0.27	1.3345 ± 0.0014	12.11 ± 0.46	−0.54 ± 0.25
C	38.95 ± 0.15	43.11 ± 0.15	1.1068 ± 0.0016	16.49 ± 0.33	−0.65 ± 0.71
D	42.25 ± 0.18	44.25 ± 0.24	1.0473 ± 0.0075	18.11 ± 0.59	−0.68 ± 0.42
E	31.77 ± 0.48	40.88 ± 0.16	1.2867 ± 0.0036	15.22 ± 0.19	−0.60 ± 0.32
F	72.36 ± 0.28	61.44 ± 0.09	0.8490 ± 0.0018	18.35 ± 0.26	−0.67 ± 0.11
G	66.85 ± 0.56	58.26 ± 0.42	0.8715 ± 0.0052	15.60 ± 0.17	−0.59 ± 0.24
H	76.36 ± 0.38	64.79 ± 0.37	0.8484 ± 0.0036	19.88 ± 0.24	−0.70 ± 0.85
I	79.71 ± 0.03	66.25 ± 0.03	0.8311 ± 0.0078	21.35 ± 0.07	−0.72 ± 0.41
J	73.08 ± 0.34	62.57 ± 0.14	0.8561 ± 0.0096	17.85 ± 0.14	−0.68 ± 0.30
K	83.11 ± 0.09	69.77 ± 0.07	0.8394 ± 0.0014	22.34 ± 0.41	−0.71 ± 0.55
L	79.22 ± 0.18	68.11 ± 0.75	0.8597 ± 0.0035	19.95 ± 0.17	−0.66 ± 0.47
M	90.90 ± 0.27	75.37 ± 0.14	0.8291 ± 0.0028	25.33 ± 0.78	−0.74 ± 0.15
N	93.58 ± 0.14	76.11 ± 0.24	0.8133 ± 0.0051	30.14 ± 0.22	−0.79 ± 0.03
O	82.04 ± 0.17	72.18 ± 0.07	0.8798 ± 0.0049	24.11 ± 0.28	−0.70 ± 0.17

3.2.3. Emulsion Stability (ES)

The main reason for emulsion instability is creaming, which is usually the result of structural and physical differences between two immiscible liquids [2]. The stability of emulsions during various processes, especially storage, is one of the methods used to determine their quality. In fact, more stability somehow indicates the high quality of the produced emulsion. The stability of an emulsion can be defined as its ability to

withstand various physical changes, especially creaming [37,38]. ES was computed (by measuring the creaming value) in days 7, 14, 21 and 28 of storage at room temperature (Table 6 represents the ES of samples). As indicated in Table 6, by treating emulsions with microwaves and ultrasound, the stability of samples was significantly raised, which might be associated with the elevation of continuous phase viscosity. Sometimes it is possible that weak gel properties in the emulsion appear as a result of the interaction between the oil molecules in the emulsion, which leads to creaming, but in the present study, what is effective in the stability of emulsions is an acceptable and effective interaction between utilized biopolymers. In other words, elevation in viscosity was a main parameter in emulsion stabilization [11,39,40]. Samples with a mixture of hydrocolloids (except the samples with more XG in their formulation) had more stability than samples with only one hydrocolloid. Among the emulsions, those with a higher ratio of FG in the formulation, had higher stability values in comparison with XG. Therefore, it can be concluded that, FG had more impact on ES, which was in agreement with the results obtained in previous sections. Samples N (75FG25XG) and B (100XG) had the highest (99.97% in initial day and 60.37% after 28 days) and lowest stability (97.12% in initial day and 35.98% after 28 days) of the emulsions.

Table 6. Stability of produced emulsions.

Samples	First Day	After 7 Days (%)	After 14 Days (%)	After 21 Days (%)	After 28 Days (%)
A	97.25 ± 0.32	93.25 ± 0.11	71.36 ± 0.06	56.24 ± 0.26	40.32 ± 0.14
B	97.12 ± 0.45	91.48 ± 0.74	68.11 ± 0.35	48.32 ± 0.11	35.98 ± 0.22
C	98.11 ± 0.12	95.32 ± 0.66	75.89 ± 0.14	60.35 ± 0.25	46.14 ± 0.66
D	98.65 ± 0.37	95.88 ± 0.03	78.96 ± 0.12	64.88 ± 0.31	48.25 ± 0.19
E	97.85 ± 0.28	93.48 ± 0.19	72.65 ± 0.48	59.08 ± 0.15	42.36 ± 0.77
F	98.26 ± 0.07	94.11 ± 0.25	77.35 ± 0.64	63.11 ± 0.19	45.34 ± 0.21
G	97.55 ± 0.64	92.13 ± 0.07	73.65 ± 0.11	52.30 ± 0.05	38.11 ± 0.45
H	98.85 ± 0.18	96.11 ± 0.09	79.25 ± 0.48	67.25 ± 0.16	49.37 ± 0.33
I	98.97 ± 0.09	96.85 ± 0.14	81.58 ± 0.79	69.88 ± 0.91	51.25 ± 0.87
J	98.08 ± 0.34	95.90 ± 0.26	77.25 ± 0.56	61.25 ± 0.48	45.22 ± 0.54
K	99.25 ± 0.74	95.92 ± 0.24	81.65 ± 0.55	70.35 ± 0.05	50.37 ± 0.18
L	98.90 ± 0.18	93.25 ± 0.78	75.88 ± 0.07	61.95 ± 0.18	40.55 ± 0.26
M	99.49 ± 0.52	97.77 ± 0.01	83.11 ± 0.91	71.54 ± 0.47	56.75 ± 0.28
N	99.97 ± 0.05	97.98 ± 0.63	87.12 ± 0.15	78.35 ± 0.26	60.37 ± 0.19
O	99.21 ± 0.36	95.98 ± 0.44	81.34 ± 0.36	70.11 ± 0.09	52.36 ± 0.42

4. Conclusions

In the current research, the effects of two hydrocolloids (FG (as a known traditional galactomannan) and XG) treated with microwaves and ultrasound on the rheological aspects of O/W emulsions were determined. The apparent viscosities of the emulsions were diminished by elevating the shear rate that authenticated the shear-thinning behavior of all samples. The strain and frequency sweep results indicated that emulsions treated with microwaves and ultrasound demonstrated weak gel-like behavior (by investigating the G', G'', tanδ and η^* values).

As a conclusion, the synergistic and acceptable interaction between two biopolymers (which could be attributed to strong intermolecular bonds and the effect of microwave and ultrasound treatment) resulted in the enhancement of various rheological parameters, particularly apparent viscosity, which is a vital parameter. The same result was also observed for emulsion stability; in emulsions with two hydrocolloids (especially in samples treated with ultrasound) the emulsion stability values of the samples were higher than those with only one hydrocolloid. Additionally, fenugreek galactomannan had more impact on this parameter in comparison with xanthan gum.

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