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Research Article

Rheological Properties of Oil-in-Water Emulsions Stabilised with Brown Bambara Groundnut Protein

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Abstract

There has been ongoing research on the functionality of legume protein in food, however, there is insufficient knowledge of the potential of brown bambara groundnut (BBGN) protein isolates and its effect on the rheological characteristics of oil-in-water (O/W) emulsions .The aim of this study was to investigate the rheological properties of O/W emulsions stabilised with BBGN protein isolates. Different compositions: Low protein emulsion (6% protein, 39% oil, 55% water), medium protein emulsion (8.14% protein, 36.63% oil 55.23% water) and high protein emulsion (15% protein, 30% oil, 55% water) were formulated. All emulsions exhibited shear thinning with the Hershel Bulkley model being the best fit model. The high protein emulsion had the longest linear viscoelastic region. Increasing the frequency increased the elasticity of the O/W emulsions. Refrigeration temperature enhanced the stability of the O/W emulsions.

Keywords: Groundnut; O/W Emulsions; Protein

Introduction

Bambara groundnut protein has the potential to stabilise oil-in-water (O/W) emulsions as it has emulsifying properties [1]. Different protein concentrations can affect the nature of the O/W emulsions hence rheology can be used to determine the structural configuration and interaction of the components involved [2]. Rheology is the study of the response of a material to applied force through deformation or flow [3]. As shelf-life is essential in food emulsions, their stability may be illustrated using both rotational and oscillatory rheological measurements [4]. Stability of O/W emulsions can be described by its strength as described by hydrogen bonds, hydrophobic, electrostatic and covalent bonds amongst the components [2]. The viscosity of an emulsion can describe its stability [5]. The rheological term, storage molulus (G') can describe the samples structural strength [6].

Materials and Methodology

Bambara groundnut seeds were purchased from Limpopo (Makhado) province. A Fritsch Pulverisette 19 Cutting mill was used for milling thus producing BGN flour. Sodium hydroxide and hydrochloric acid were used as pH adjusting agents. A 212 µm sieve was used to sieve the flour. An overhead stirrer was used for mixing distilled water and BGN flour during protein extraction. An Ortoalresa Digtor 21 centrifuge was used for concentrating solids from liquid. Trays were used for protein drying. A porcelain mortar and pestle were used for grinding the protein. A D-lab homogeniser was used for homogenising protein, oil and water. A Discovery Hybrid rheometer using a concentric cylinder geometry (DIN rotor and cup with specifications: bod diameter: 28mm; length 42 mm; cup diameter 30.4 mm) was used to conduct rheology tests on O/W emulsions.

- **Protein extraction:** A mixture of BGN flour and distilled water at a ratio of 1:10 was stirred for 5 minutes. 1M of NaOH was used to adjust the pH of the mixture to 8. The mixture was centrifuged at 3500g for 30 minutes. 1M HCl was used to adjust the pH of the supernatant to 4 and stirred for 1 hour followed by centrifugation at 3500g for 30 minutes. The solids obtained was the protein which was spread out on a tray to air dry for 48 hours. Dried protein was grinded into fine powder using a porcelain mortar and pestle.

- **Emulsion Formulation:** A speed of 10 000 rpm was used to homogenise distilled water and protein for 1 minute. The protein solution was homoged at the same speed for 5 minutes. Different compositions: Low protein emulsion (LPE) (6% protein, 39% oil, 55% water), medium protein emulsion (MPE) (8.14% protein, 36.63% oil 55.23% water) and high protein emulsion (HPE) (15% protein, 30% oil, 55% water) were formulated.

- Rotational and oscillatory tests were conducted to determine the flow and viscoelastic behaviour of O/W emul-

sions respectively. The oscillatory tests included the amplitude, frequency, temperature and time sweep tests. Before oscillatory tests were conducted, the samples were equilibrated for 10 minutes [7].

- Rotational Steady State Test: The steady-state test was conducted by firstly subjecting samples under a constant shear rate of 100 s-1 for 10 minutes. After that, the shear rate was varied from 10^{-2} to 1000 s^{-1} at 25°C. The apparent viscosity was obtained from the relationship between the shear stress versus shear rate.

The data obtained from the rotational steady state test was fitted to conventional time- independent models (Power law (equation 2.1), Herschel-Bulkley (equation 2.2), Casson (equation 2.3) and Bingham (equation 2.4)) using Matlab 2017 where a nonlinear regression tool was used and the Levenberg- Marquardt algorithm was adopted as the optimisation tool in order to calculate the R-squared, sum of square error (SSE) and root mean square error (RMSE) [8].

$\tau = K\dot{\gamma}^n$ Equation 2.1

$\tau = \tau_0 + K\dot{\gamma}^n$ Equation 2.2

$\tau^{0.5} = (\tau_0^C)^{0.5} + (\eta^C)^{0.5}\dot{\gamma}^{0.5}$ Equation 2.3

$\tau = \tau_0^B + \eta^B \dot{\gamma}$ Equation 2.4

Where τ is stress (Pa), K consistency coefficient ($Pa.s^n$), $\dot{\gamma}$ shear rate s^{-1}, n flow behaviour index (dimensionless) (where n = 1 represents Newtonian behaviour, n > 1 shear thickening (dilatant) and n < 1 shear thinning (pseudoplastic)), τ_0 yield stress (Pa), $\dot{\gamma}$ shear rate s^{-1}, $\tau 0^B$ Bingham yield stress (Pa), η^B Bingham viscosity (Pa.s), $\tau 0^C$ Casson yield stress (Pa) and η^C Casson viscosity (Pa.s).

Amplitude oscillatory sweep test (Strain sweep)

Strain sweep test was conducted at a strain range of 0.1 to 1000% under a fixed frequency of 1 Hz. The linear viscoelastic region (LVR) and the transition point (where G' = G'') was extracted from the plot.

Oscillatory frequency sweep test

The frequency sweep test was conducted at a frequency range of 0.01 to 100 Rad/s [9] at a fixed temperature 25°C and a constant strain of 0.2% obtained from the amplitude sweep test. The G' and G'' were obtained at the varying frequencies demonstrating the effect of frequency on the rheological properties of the O/W emulsions.

Oscillatory temperature sweep test

The O/W emulsions were subjected to a temperature of 0 to 40°C at a constant strain of 0.2% and a constant frequency of 1 Hz to determine the effect of storage temperature.

Oscillatory time sweep test

The effect of time on the rheological properties of the O/W emulsions were determined by subjecting the samples for 1 hour at a constant strain of 0.2%, frequency of 1 Hz and temperature of 25°C.

Results and Discussion

Effect of Protein, Oil and Water on the Viscosity of O/W Emulsions

Figure 1 illustrate the behaviour of the emulsion's viscosity with the change in shear rate. At the lowest shear rate of 0.01 s^{-1} is where the least structural disturbance occurs. At this shear rate, the viscosity increased as the protein concentration increased. Iqbal., *et al.* [10] investigated how egg white protein affects the stability of O/W emulsions and concluded that the increase in protein concentration increased emulsion viscosity which is a similar to the findings of this study. All emulsions exhibited shear thinning a result of weakening of bonds as the shear rate increased. Shear thinning was also observed in the study of Iqbal., *et al*. [10]. The LPE had the least viscosity of 90.93 Pa.s followed by that of the MPE (151.52 Pa.s) then the HPE (232.33 Pa.s) at a shear rate of 0.01 s^{-1}. Between a shear rate 0.1 and 1 s^{-1}, the emulsions started experiencing infinite shear rate viscosity [11] where breaking of bonds could not take place any longer.

Figure 1: The relationship between viscosity and shear rate.

The model parameters of the Power Law, Herschel- Bulkley, Bingham and Casson models are described in Table 1, 2, 3 and 4, respectively. The Power Law and Herschel- Bulkley models confirms that all emulsions experienced shear thinning as n (flow behaviour index) demonstrated by both the models was less than 1. All emulsions had yield stress where the Bingham model illustrated the HPE to have a higher yield stress (3.99 Pa) followed by the MPE (3.23 Pa) then the LPE (1.48 Pa). The Casson model also illustrates the HPE to have a higher yield stress (2.13 Pa) followed by the MPE (1.73 Pa) then the LPE (0.21 Pa).

The Casson viscosity was higher than the Bingham viscosity for all emulsions. As the HPEs were more viscous than the MPEs fol-

lowed by the LPEs, it was expected that the consistency coefficient would follow that trend. This trend was demonstrated by the consistency coefficient of both the Power Law and Herschel-Bulkley models. According to the Power Law and Herschel- Bulkley model, the HPE had a consistency coefficient of 2.48 and 1.65 Pa.s^n , the MPEs 1.67 and 0.56 Pa s^n , the LPE 0.39 and 0.20 Pa s^n respectively. All emulsions were best fitted using the Hershel-Bulkley model as the data using this model had the lowest RMSE and SSE and a high R^2 compared to those of the Power Law, Bingham and Casson models. According to Izidoro., *et al.* [12], the characteristics of mayonnaise and salad dressing can be described using the Power Law, Herschel- Bullkley and Casson models. As the Herschel-Bullkley model was the best fit model for all the emulsions, BBGN protein could be used to stabilise these products.

Emulsion Category	Protein %	Oil %	Water %	n	k	R^2	Adjusted R^2	SSE	RMSE
LPE	6.00	39.00	55.00	0.5704	0.3889	0.9864	0.9858	8.648	0.6003
MPE	8.14	36.63	55.23	0.3673	1.6710	0.9659	0.9645	26.240	1.0460
HPE	15.00	30.00	55.00	0.3294	2.4800	0.9776	0.9766	24.600	1.0120

Table 1: Power Law Model Parameters.

Emulsion Category	Protein %	Oil %	Water %	n	k	τ	R^2	Adjusted R^2	SSE	RMSE
LPE	6.00	39.00	55.00	0.6658	0.1986	0.8061	0.9986	0.9985	0.9055	0.1984
MPE	8.14	36.63	55.23	0.5222	0.5639	1.8500	0.9979	0.9978	1.5820	0.2622
HPE	15.00	30.00	55.00	0.3855	1.6520	1.1130	0.9831	0.9817	18.5000	0.8968

Table 2: Herschel-Bulkley Model Parameters.

Emulsion Category	Protein %	Oil %	Water %	η	τ	R^2	Adjusted R^2	SSE	RMSE
1 LPEs	6.00	39.00	55.00	0.02107	1.484	0.9630	0.9615	23.520	0.9900
8 MPEs	8.14	36.63	55.23	0.02262	3.226	0.9151	0.9116	65.410	1.6510
4 HPEs	15.00	30.00	55.00	0.02557	3.992	0.8230	0.8156	194.000	2.8430

Table 3: Bingham Model Parameters.

Emulsion Category	Emulsion	Protein %	Oil %	Water %	η	τ	R^2	Adjusted R^2	SSE	RMSE
LPE	1	6.00	39.00	55.00	0.5882	0.21010	0.9821	0.9813	11.410	0.6895
MPE	8	8.14	36.63	55.23	0.6526	1.73300	0.9976	0.9975	1.855	0.2780
HPE	4	15.00	30.00	55.00	0.7677	2.12900	0.9710	0.9698	31.800	1.1510

Table 4: Casson Model Parameters.

Effect of strain on the viscoelastic properties of O/W emulsions

Figure 2 describes the behaviour of the storage and loss modulus over a strain range. All emulsions initially had a LVR which showed stability at low strains and at a particular point both G' and G'' deviated and decreased. The emulsions had viscoelastic properties however the composition of protein, oil and water of had an effect on the moduli.

During the LVR, the moduli of the HPE was higher followed by the MPE and the LPE indicating that the HPE was more stable. The elasticity of the LPE was seen to be lower compared to the HPE as fewer oil and water molecules were held together per unit area by the BBGN protein. The HPE had higher loss modulus than the MPE and LPE during the LVR probably due to higher internal friction of molecules. The results of this study is similar to the work performed by Bengoechea., *et al.* [13] and Primozic., *et al.* [14]. Bengoechea., *et al.* [13] performed oscillatory tests on O/W emulsions stabilised by egg protein and reported that the emulsions had viscoelastic properties and showed that emulsions had an increasing trend of G' and G'' as protein concentration increased. Primozic., *et al.* [14] performed a strain amplitude sweep test on O/W na-

Figure 2: The effect of strain on viscoelastic properties of O/W emulsions.

noemulsions stabilised by lentil protein isolate and illustrated the dominance of the elastic portion before the crossing of G' and G''. They also reported an increasing trend of G' and G'' as the protein concentration increased.

As the HPE was more viscous than the MPE and the LPE, it was expected that it would have a longer LVR and that was observed in this study. The HPE had a LVR ending at a strain of 1.02% followed by the MPE (0.64%) and the LPE (0.4%). The same trend was expected to occur for the point where G' and G'' are equal however that was not the case as the LPE had a crossing of G' and G'' at a higher strain (probably due to wall slip) than that of the HPE and the MPE.

Effect of frequency on the viscoelastic properties of O/W emulsions

A strain of 0.2% was chosen to perform the frequency sweep as all emulsions were still at their LVR at this strain. Peng., *et al.* [15] performed a frequency sweep test on whipped cream at a range of 1 to 100 Hz at 0.5% strain where the storage and loss modulus illustrated an increasing trend with increasing frequency. This study demonstrated the same trend. At low frequencies, all emulsions had a low moduli which increased at high frequencies.

According to De Vicente [16], at high frequencies, there is insufficient time to enable polymer chains to disentangle while they have enough time to disentangle at low frequencies. According to Mezger [17], at high frequencies, polymer entanglements are less mobile while at low frequencies, they are more mobile. Therefore, at high frequencies the elasticity increased as the polymer chains were probably less mobile and unable to disentangle to initiate deformation. The storage modulus had a steep increase at the beginning of the test. This may have occurred due to combination of the increase in frequency and the energy that the molecules still possessed immediately after homogenisation that resulted in bond formation. From a frequency of 10 rad/s, the elasticity increased gradually as the molecules might not have contained much energy as they initially had. All emulsions had a very gradual increase in the loss modulus. The moduli of the HPE was greater than that of the MPE followed by the LPE. Bengoechea., *et al*. [18] and Puppo., *et al.* [19] also reported the increase in the elasticity with increasing frequency in O/W emulsions stabilised by soy protein. According to Ikeda and Nishinari [20], weak gels are characterised by tanδ > 0.1. Therefore, all emulsions in this study are weak gels as their $\tan\delta\ (\frac{G''}{G'})$ was greater than 0.1.

Effect of temperature on the viscoelastic properties of O/W emulsions

Figure 4 shows the effect of temperature on viscoelastic properties of O/W emulsions at refrigeration temperature, room temperature and at higher temperatures up until 40°C.

Between 10 to 40°C, the G' and G'' curves were horizontal and almost parallel. Between the temperatures 10 to 35°C, which resembles cold to room temperature, there was no negative effect on the structure of the emulsions since there was no decrease in elasticity or increase in the loss modulus thus demonstrating stability of emulsions under such conditions.

Figure 3: Effect of frequency on the moduli of O/W emulsions.

Figure 4: Effect of temperature on the moduli of O/W emulsions.

The temperatures 35 to 40°C resembled temperatures above room temperatures. Stability was maintained between these temperatures as the elastic portion remained dominant over the viscous portion. All the emulsions were able to withstand the higher temperature which did not break the bonds between the molecules.

The refrigeration to cold temperatures was represented by temperatures from 5 to 10°C. There was a significant decrease in G' in the elasticity of the emulsions from 5 to 10°C. The decrease in elasticity between these temperatures may illustrate crystalline breakdown. Since elasticity was greater at refrigeration temperature than at room the temperature, this may suggest that the stability of the emulsions can be enhanced at refrigeration temperature.

Effect of time on the viscoelastic properties O/W emulsions

There were structural changes that occurred an one hour after homogenisation as the elasticity increased. After homogenisation due to continuous mobility of the molecules, collisions could have occurred resulting in bond formation thus increasing the elasticity. Polymer cross-linking could have enhanced elasticity as well. Since homogenisation caused samples to be warm, the cooling of the emulsions with time could have enable molecules to move closer together, strengthening bonds and increasing the elasticity. The loss modulus had a very gradual increase over the period of one hour.

Figure 5: Effect of time on the moduli of O/W emulsions.

Conclusion

O/W emulsions stabilised by BBGN protein exhibited shear thinning and viscoelastic properties. Increasing protein concentration increased the LVR of the O/W emulsions. Increasing frequency increased the elasticity of O/W emulsions. Refrigeration temperature enhanced stability of O/W emulsions.

Bibliography

1. Adebowale YA., *et al*. "Protein isolates from Bambara groundnut (Voandzeia Subterranean L.): Chemical characterization and functional properties". *International Journal of Food Properties* 14.4 (2011): 758-775.

2. Geremias-Andrade I., *et al*. "Rheology of Emulsion-Filled Gels Applied to the Development of Food Materials". *Gels* 2(2016): 22.

3. Prasad V., *et al*. "Confocal microscopy of colloids". *Journal of Physics Condensed Matter* 19.11 (2007): 1-25.

4. Tadros T. "Application of rheology for assessment and prediction of the long-term physical stability of emulsions" (2004).

5. Yani S., *et al*. "Evaluation of viscosity and pH on Emulsions of Virgin Coconut Oil Beverages". *IOP Conference Series: Earth and Environmental Science* 175(2018).

6. Yang, M., *et al*. "Properties and microstructure of transglutaminase-set soy protein-stabilized emulsion gels". *Food Research International* 52 (2013): 409-418.

7. Adeyi O., *et al*. "Emulsion Stability and Steady Shear Characteristics of Concentrated Oil- in- Water Emulsion Stabilized by Gelatinized Bambara Groundnut". *Asian journal of Chemistry* 26.16 (2014): 4995-5002.

8. Khalil M and Mohamed Jan B. "Viscoplastic modeling of a novel lightweight biopolymer drilling fluid for underbalanced drilling". *Industrial and Engineering Chemistry Research* 51.10 (2012): 4056-4068.

9. Kutz M. "Handbook of Farm, Dairy and Food Machinery Engineering". 2nd ed. USA: Academic Press (2013).

10. Iqbal S., *et al*. "Impact of egg white protein on the quality and stability of corn oil-in-Water emulsion". *Journal of the Chemical Society of Pakistan* 39 (2017): 911-918.

11. Gaonkar AG. "Ingredient Interactions: Effects on Food Quality". USA: Marcel Dekker (1995).

12. Izidoro DR., *et al*. "Rheological properties of emulsions stabilized by green banana (Musa cavendishii) pulp fitted by power law model". *Brazilian Archives of Biology and Technology* 52.6 (2009): 1541-1553.

13. Bengoechea C., *et al*. "Influence of semi continuous processing on the rheology and droplet size distribution of mayonnaise-like emulsions". *Food Science and Technology International* 15 (2009): 367-373.

14. Primozic, M., *et al*. "Effect of lentil proteins isolate concentration on the formation, stability and rheological behavior of oil-in-water nanoemulsions". *Food Chemistry* 237 (2017): 65-74.

15. Peng F., *et al*. "Physical, textural, and rheological properties of whipped cream affected by milk fat globule membrane protein". *International Journal of Food Properties* 21.1 (2018): 1190-1202.

16. De Vicente J. Rheology. Croatia: InTech (2012).

17. Mezger TG. "The Rheology Handbook: For Users of Rotational and Oscillatory Rheometers". 2nd ed. Germany: Vincentz Network (2006).

18. Bengoechea C., *et al*. "Rheology and microstructure of gluten and soya- based o/w emulsions". *Rheologica Acta* 46 (2006): 13-26.

19. Puppo MC., *et al*. "Rheological properties of emulsions containing modified soy protein isolates". *Journal of the American Oil Chemists' Society* 80 (2003): 605-611.

20. Ikeda S and Nishinari K. "'Weak gel'-type rheological properties of aqueous dispersions of nonaggregated κ-carrageenan helices". *Journal of Agricultural and Food Chemistry* 49 (2001): 4436-4441.

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