Investigation of the Emulsifying Properties of Bambara Groundnut Flour and Starch

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Abstract—The current desire in food and industrial emulsification is the use of natural emulsifiers. Bambara groundnut flour (BGNF) and its starch (BGNS) will serve both emulsifying and nutritional purposes if found suitable. This current study was aimed at investigating the emulsifying properties of BGNF/BGNS. BGNS was extracted from the BGNF. Emulsions were prepared using a wide range of flour-oil-water and starch-oil-water composition as generated through the application of Response Surface (D-optimal) design. Prepared emulsions were investigated for stability to creaming/sedimentation (using the kinetic information from turbiscan) and flocculation/coalescence (by monitoring the droplet diameter growth using optical microscope) over 5 days. The most stable emulsions (one BGNF-stabilized and the other BGNS-stabilized) were determined. The optimal emulsifier/oil composition was 9g/39g for BGNF and 5g/30g for BGNS. The two emulsions had only 30% and 50% growth in oil droplet diameter respectively by day 5, compared to over 3000% in the unstable ones. The BGNF-stabilized emulsions were more stable than the BGNS-stabilized ones. Emulsions were successfully stabilized with BGNF and BGNS.

Keywords—Bambara groundnut, coalescence, creaming, emulsification, emulsion, emulsion stability.

I. INTRODUCTION

THERE is growing interest by the food, pharmaceutical and cosmetic industries in replacing synthetic emulsifiers with natural ones. This is because natural emulsifiers are more biocompatible and could demonstrate satisfactory amphiphilic properties [1], [2]. The search for such natural products has yielded results with the use of the products of some leguminous plant like soybean and flaxseed in emulsion preparations [3]. The search for emulsifiers among natural plant/food products is particularly desirable due to their often non-toxic nature, affordability and availability, and their already established food and medicinal uses [3].

Bambara groundnut [BGN] (*Vigna subterranea*), a leguminous plant which belongs to the family fabaceae is an indigenous Africa plant that has been cultivated all over sub-Saharan Africa [4]. As important as BGN is as a source of food in many African communities, very limited studies have been conducted to assess its potential benefit to the food and pharmaceutical industries. Although there is no documented

study indicating the emulsifying capacity of Bambara groundnut (BGN), its rich blend of protein and carbohydrate suggests its potential as an emulsifier.

In a study to analyze its nutritional composition, Doku [5] reported relatively high lysine content while another study by Amarteifio and co-workers [6] showed a breakdown of 53.1% carbohydrate, 17.4% protein, 6.1% fat, 6.1% fiber, and 3.4% ash, as well as low levels of calcium, iron, sodium and potassium. The protein in BGN is said to be richer as it has been reported to contain more essential amino acid (methionine) than any other legume [7]-[9]. In addition to the relatively high protein content in BGN, the total nutritional composition reflects an excellent balance unusual in single plant products. A number of food, pharmaceutical, and industrial products are prepared as emulsions [10]. Thus, BGN flour (BGNF) and starch (BGNS, if found suitable as emulsifier in food products, could impact added nutritional advantage [11].

In order to establish the suitability of a prospective emulsifier for emulsion preparation, various stability tests are have evolved. A number of instrumental methods have been developed to characterize the physicochemical properties of emulsions. Such instruments are employed to extract information on the change in droplet concentration, and size with time. The most commonly used instrumentations are based on the principle of light scattering in which a monochromatic beam of near infrared light is directed through an emulsion placed in a vertical flat-bottomed glass tubes [12], [13]. Thus, the percentage of transmitted or backscattered light measured as a function of the height of the emulsion provides an indication of changes in droplet concentrations along the height and can be used to determine creaming and/or sedimentation [14]. Due to the commercial availability of fully-automated analytical instruments based on this principle, optical profiling technique is one of the very popular ways for industrial characterization of gravitational separation in emulsions before such instability is visible to the eye [15]. One of such automated instrument (used in this study) is Turbiscan® which was employed in this study. In addition the growth of emulsion droplet through optical microscopy can provide indication of emulsion stability.

The aim of this study was to investigate the emulsifying properties of BGNF and BGNS and to determine optimal concentration required to stabilize typical oil-in-water emulsion.

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II. MATERIALS AND METHODS

A. Materials

Bambara groundnut was obtained from Triotrade, Gauteng CC., South Africa. Sunflower oil was purchased from local supermarket in Cape Town. All other materials and equipment were obtained from the Departments of Chemical Engineering and Food Technology laboratories of the Cape Peninsula University of Technology. Chemical reagents used were of analytical grade. The major equipment used in this study were turbiscan® (Turbiscan MA 2000, Formulaction, Toulouse, France), homogenizer (Ultra-Turrax T25, Janke and Kunkel, Staufen, Germany) and a digital research microscope (Ken-a-vision, Kansa city, MO, USA).

B. Preparation of Bambara Groundnut Flour

Bambara groundnut was milled using a hammer mill (Trapp TRF 400, Animal ration shredder/hammer mill foliage, Jaraqua do sul-sc, Brasil) to produce the Bambara groundnut flour (BGNF). The flour was sieved by passing through 250μ mesh for homogeneity.

C. Isolation of Starch from BGN Flour

The method of Adebowale and co-workers [16] was employed with modifications to isolate starch from the BGN flour. A typical extraction procedure involved the mixing of weighed BGN flour with water (1:10 w/v) at room temperature for 1h. Thereafter, the mixture was allowed to stand for 5h followed by centrifugation (3,500 xg, 30min). The supernatant was discarded and the residue was subjected to similar procedure, first with water containing 2% w/v NaCl (10min mixing and 12h standing); and then 0.03M NaOH (10min mixing and 12h standing). The resultant residue was re-constituted in water and passed through a 75μm sieve to remove the fiber. The sieved mixture which contained the pure starch was then left for 2h to sediment after which the supernatant was decanted. The residue was air-dried (at room temperature) to yield the BGN starch (BGNS).

D. Determination of Oil:Water:BGN Flour/Starch Ratio in Emulsions

The D-Optimal response surface methodology was used to generate a range of emulsions using BGNF/S and oil as variables [17] (Table I). A total number of 12 emulsions templates were thus generated with duplicates. Following this design, the emulsions were prepared by gelatinizing the emulsifier (BGNF or BGNS) (quantities as designed in Table I) in sufficient water to make a 100g mixture. Gelatinization was achieved by heating and stirring the BGNF/S-water mixture on a heater equipped with magnetic stirrer for 10min [18]. The resultant gel was then left to cool to room temperature before the oil was added. The addition of oil was immediately followed by homogenization using high-speed homogenizer (IKA Ultra-Turrax® T25, Digital Janke and Kunkel, Staufen, Germany) set at 15000 rpm for 10min. The quanlity of the emulsions were accessed using migration rate and droplet size.

TABLE I
PROCESS VARIABLES USED FOR THE D-OPTIMAL RESPONSE SURFACE DESIGN FOR BGNF/S EMULSION COMPOSITION [a, b]

Variable [b]	Symbol	Coded values[a]		
		-1	0	+1
Flour (g)	X_1	2	6	10
Oil (g)	X_2	5	30	55

[a] Transformation of coded variable (x_i) to uncoded variable (X_i) levels could be obtained from $X_1 = 4x_1 + 6$; $X_2 = 25x_2 + 30$,
[b] This table was the same for BGNS except that the BGNS concentrations were halves of the corresponding BGNF.

E. Migration/Creaming Rate Determination

The two major destabilization phenomena affecting the homogeneity of emulsions are droplet migration (creaming, sedimentation) and particle size variation or aggregation (coalescence, flocculation) [14]. Droplet migration was investigated using the turbiscan, while size variation was monitored using an optical microscope.

The emulsion sample (7mL) was transferred into the tubes (Turbiscan cell) and the ΔBS% was recorded every 1min over 20min, repeated daily for 5 days. Observations for creaming and sedimentation were made and the data were computed using the migration software equipped in the turbiscan [14].

The migration rate was computed by following the migration front using the "Migration" software, available systematically on the Turbiscan. Typically, the slope of the cream peak thickness kinetics was first identified. The linear portion of this slope was zoomed and copied into the 'migration' from where the migration rate (equivalent to the creaming index) is computed. The migration rate of creamed emulsion was used as the negative control. Percentage instability was calculated relative to the control [19].

F. Determination of Optimal BGN Flour/Starch for Optimal Emulsion

The migration data was fitted to quadratic response model and analysis of variance was used to establish the effect of flour/starch and oil on the emulsion migration rate. The optimum flour/starch emulsion was determined using the numerical optimization algorithm in Design Expert software [20]. The goal of the optimization was to minimize migration rate within the range of flour/starch and oil in the emulsion.

G. Droplet Size Determination

Changes in droplet size and concentration were monitored using micrographs obtained with the aid of the Ken-a-vision digital research microscope. Micrographs were obtained by placing a drop of the emulsion on a microscope slide. The micrographs were then displayed on the computer screen through the attached Applied VisionTM software. The mean diameter of representative droplets (50 per micrograph) was then obtained by using the measurement calibration in the Applied VisionTM Software.

H. Data Analysis

Analysis of variance (ANOVA) was used to establish mean differences between treatments at 5% probability. Duncan multiple range test was used to separate means where significant difference existed.

III. RESULT AND DISCUSSION

A. Bambara Groundnut Starch Yield

The process of starch extraction was successful, yielding 31.4% starch. This is lower than the yield (37.5%) earlier reported [16] and the varieties used might have been different. The starch yield from the current extraction represents the starch after drying. The starch obtained was powdery, whitish, tasteless and odourless.

Earlier studies have reported varied carbohydrate composition of varieties of BGN. Sirivongpaisal [21] reported 11.4% protein and 53.1% carbohydrate in BGN while Eltayeb [22] reported 17.7% protein and 86% total carbohydrate (including crude fibre and starch). There is variation in the nutritional composition of BGN which could explain the variation in starch content, reported in this work and that of Adebowale and co-workers [16].

B. Effect of Oil and BGNF on the Migration Rate and Droplet Size of O/W Emulsions

Table II provides the details of the effect of BGNF and oil concentration on migration rate (MR) and droplet diameter of BGNF stabilized emulsions on the first day. Emulsions with 2% w/v BGNF and varying amount of oil 5% w/v, 30% w/v and 55% w/v oil showed migration rates in increasing order: 0.2350 ± 0.0040 mm/min, 0.0300 ± 0.0002 mm/min, and 0.0580 ± 0.0015 mm/min respectively. This suggests that the higher the oil concentration, the higher the instability. It may also suggest that the concentration of the emulsifier is too low to emulsifying such oil:water proportion thereby encouraging creaming, which is the movement of the oil droplets from the bottom of the turbiscan tube to the top (Fig. 1). Furthermore, the lowest values of MR (0.0043 ± 0.0002 mm/min, 0.0004 ± 0.0004 mm/min and 0.0006 ± 0.0002 mm/min), were observed in the emulsion with highest BGNF (10% w/v). When compared within the group (10% w/v BGNF, and varying oil concentration – 5% w/v, 30% w/v and 55% w/v), the most stable emulsion was produced by the lowest oil composition. This could be due to the fact that the concentration of the BGNF was high enough to hold the water and oil together resulting in slow migration of the droplet particles.

In general, the emulsion MR ranges from 0.2350 ± 0.0040 mm/min for the lowest BGNF (2% w/v) to 0.0043 ± 0.0002 mm/min for the highest BGNF (10% w/v) concentration. This implies that the higher the BGNF concentration, the more stable the emulsion. Similar trend was observed with the droplet diameter. Emulsions with the lowest BGNF concentration (2% w/v) but varying amount of oil have the highest ranges of droplet diameters 0.0300 ± 0.0020μm to 0.0720 ± 0.00030μm. The lowest droplet diameter of 0.0004 ± 0.0004μm was obtained from the emulsion with highest BGNF concentration (10% w/v). Data from the photomicrographs presented in Fig. 2 show the decreasing droplet size with increasing concentration of BGNF. Similar observation has been reported by Zinoviadou and co-workers [23] in their work to determine the properties of emulsions stabilized by sodium caseinate-chitosan complex. They reported that emulsions stabilized by these complexes with increased levels of chitosan (>0.2% w/w) had a smaller average droplet size and exhibited greater stability during storage.

TABLE II
MIGRATE RATE AND DROPLET DIAMETER OF OIL-WATER EMULSION CONTAINING BGNF

Sample	Flour (% w/v)[1]	Oil (% w/v)[2]	Migration rate (MR) (mm/min)[3]	Droplet diameter(μm)[3]
1	2	5	0.2350 ± 0.0040	0.0300 ± 0.0020
2	2	30	0.0370 ± 0.0020	0.0720 ± 00003
3	2	55	0.0580 ± 0.0015	0.0720 ± 0.0003
4	6	5	0.1400 ± 0.0300	0.0120 ± 0.0001
5	6	30	0.0490 ± 0.010	0.0008 ± 0.0004
6	6	55	0.0140 ± 0.0200	0.0007 ± 0.0004
7	10	5	0.0350 ± 0.0030	0.0004 ± 0.0002
8	10	30	0.0043 ± 0.0002	0.0004 ± 0.0004
9	10	55	0.0059 ± 0.0002	0.0006 ± 0.0002

[1]Values represent the amount of flour gelatinized with sufficient water to make 100 mL
[2]Values represent the amount of oil added to the 100 g gelatinized flour
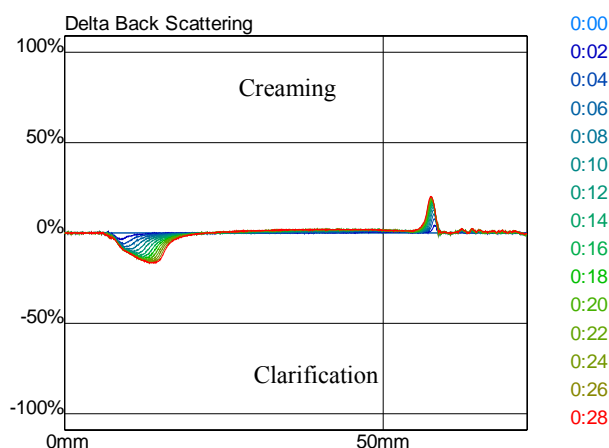[3]Values are mean ± standard deviation

Fig. 1 Turbiscan profile of emuslison containing 2% w/v BGNF and 55% w/v oil showing destabilization on day 1

C. Response Surface Model for BGNF Emulsion

The analysis of variance (ANOVA) for quadratic polynomial model for emulsion migration rate is given in Table III. The lack of fit was not significant (p > 0.05); high R2 value of 0.996 and high adjusted R2 value of 0.990 indicate that the quadratic polynomial model adequately explained the variation in the MR. Hence; the model was adequate to explore the design space. The main effect of flour and oil was significant (p < 0.05) on the migration rate, the quadratic effect of oil was significant (p < 0.05) on migration rate. However, the quadratic effect of flour was not significant (p > 0.05). There was significant (p < 0.05) interaction between the flour and oil on migration rate.
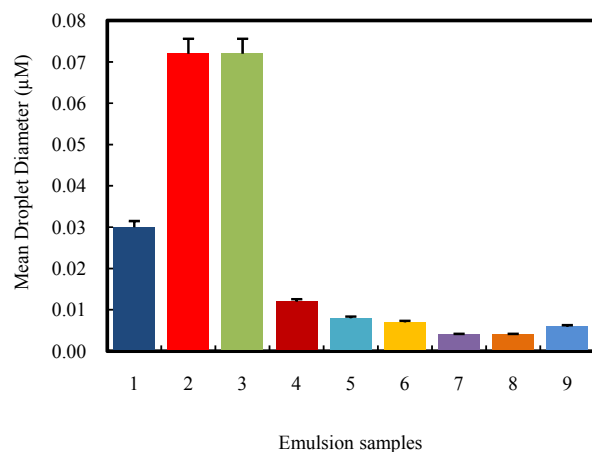
Fig. 2 The absolute mean droplet sizes of the BGNF-stabilized emulsions as observed on Day 1: 1 = 2% w/v + 5% w/v oil; 2 = 2% w/v BGNF + 30% w/v oil; 3 = 2% w/v BGNF 55% w/v oil; 4 = 6% w/v BGNF + 5% w/v oil; 5 = 6%; BGNF + 30% w/v oil; 6 = 6% w/v BGNF + 55% w/v oil; 7 = 10% w/v BGNF + 5% w/v oil; 8 = 10% w/v BGNF + 30% w/v oil; 9 = 10% w/v BGNF + 55% w/v oil

TABLE III
ANALYSIS OF VARIANCE FOR THE EFFECT OF BGNF AND OIL ON THE EMULSION MIGRATION RATE

Source	Sum of squares	df	Mean square	F-value	p-value
Model	0.071	5	0.014	186.92	
Flour	7.520E-003	1	7.520E-003	99.00	0.0006
Oil	0.012	1	0.012	164.24	0.0002
Flour * Oil	1.561E-003	1	1.561E-003	20.55	0.0106
Flour^2	5.084E-004	1	5.084E-004	6.69	0.0609
Oil^2	1.588E-003	1	1.588E-003	20.91	0.0102
Residual	3.039E-004	4	7.596E-005		
Lack of fit	3.857E-006	1	3.857E-006	0.039	0.8569
Pure error	3.000E-004	3	1.000E-004		
Cor Total	0.071	9			
R^2	0.9957				
Adj R^2	0.9904				
C.V%	10.45				

The effect of flour and oil are negative on the migration rate. This means that increasing oil or flour decreased migration rate. In other words, they cannot stabilize emulsion singly, but the interaction of both flour and oil including their quadratic effect has positive effect on the migration rate. They both encourage emulsion stability by lowering the migration rate and this can be seen from Fig. 3 where the increase in flour and oil leads to decrease in the migration rate.
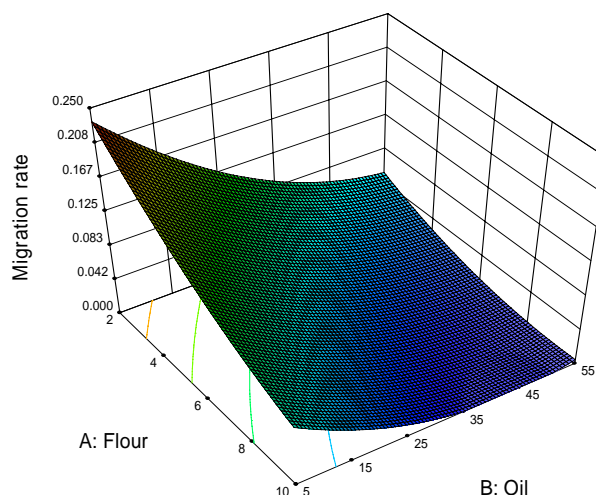

Fig. 3 The effect of BGNF (%w/v) and oil (%w/v) concentration on the droplet migration rate of the BGNF-stabilized emulsions

The relationship between the BGNF and oil with respect to migration rate is given in (1).

$$MR = 0.3315 - 0.0332\,X_1 - 7.5222 \times 10^{-3} X_2 + 2.6571 \times 10^{-4}\,X_1 X_2 + 1.0111 \times 10^{-3} X_1^2 + 5.7371 \times 10^{-5} X_2^2 \quad (1)$$

where MR = migration rate (mm/min), X_1 = BGNF (% w/v), X_2 = Oil (% w/v), $X_1 X_2$ = interactive effects of BGNF and oil, X_1^2 = quadratic effect of BGNF, X_2^2 = quadratic effect of Oil.

D. Optimum BGNF for a Stable Emulsion

Numerical optimization was used to estimate the BGNF and oil concentration that will produce minimum migration rate. The emulsion with 9% w/v BGNF and 39% w/v oil with desirability produced the optimal emulsion. This result was verified by producing the emulsion with 9% w/v BGNF and 39% w/v oil. The migration rate of the optimal emulsion was very low and physically-observed stable.

E. Effect of oil and BGNS on the Migration Rate (MR) and Droplet Size of BGNS Emulsion

The effect of BGNS on the MR and droplet size is shown in Table IV. The MR ranges from the highest value of 0.4400 ± 0.0150 mm/min for the emulsion with the lowest amount of BGNS (1% w/v) and highest oil (55% w/v) to the lowest MR value of 0.0021 ± 0.0003mm/min for the emulsion containing the highest amount of BGNS (5% w/v) and higher amount of oil (30% w/v).

The highest droplet size of 0.0700 ± 0.0003μm was observed in the emulsion containing the lowest amount of BGNS (1% w/v) and the highest amount of oil. In the same way, the lowest droplet size of 0.0100 ± 0.0002 μm was found in the emulsion with the highest BGNS concentration (5% w/v) and the higher amount of oil (30% w/v). Therefore, instability of the emulsions increased with decreasing BGNS concentration, and increasing amount of oil. Fig. 4 shows the effects of increasing BGNF concentration on droplet size. Fig.

5 provides graphical profiles of the absolute mean droplet sizes with time for BGNS-stabilized emulsions.

TABLE IV
MIGRATION RATE AND DROPLET DIAMETER OF OIL-WATER EMULSION CONTAINING BGNS

| Sample	Starch (% w/v)[1]	Oil (% w/v)[2]	Migration rate (mm/min)[3]	Droplet diameter (μm)[3]
1	1	5	0.1250 ± 0.0015	0.0240 ± 0.0002
2	1	30	0.1180 ± 0.0020	0.0254 ± 0.0003
3	1	55	0.4400 ± 0.0150	0.0700 ± 0.0003
4	3	5	0.0180 ± 0.0020	0.0130 ± 0.0004
5	3	30	0.0605 ± 0.0005	0.0380 ± 0.0004
6	3	55	0.1595 ± 0.0030	0.0450 ± 0.0004
7	5	5	0.0066 ± 0.0003	0.0110 ± 0.0001
8	5	30	0.0021 ± 0.0003	0.0100 ± 0.0002
9	5	55	0.0088 ± 0.0004	0.0120 ± 0.0003

[1]Values represent the amount of Starch gelatinized with sufficient water to make 100 mL.
[2]Values represent the amount of oil added to the 100 mL gelatinized flour.
[3] Values are mean ± standard deviation.

F. Response Surface Model for BGNS Emulsion

The analysis of variance (ANOVA) for quadratic polynomial models for the emulsion migration rate of BGNS-stabilized emulsion is given in Table V. The insignificant (p > 0.05) lack of fit in addition to the high R2 value of 0.996 and high adjusted R2 value of 0.990 indicate that the quadratic polynomial model adequately explained the variation in the MR. Hence, the model was adequate to explore the design space. The main effect of starch and oil was significant (p < 0.05) on the migration rate, the quadratic effect of oil as well as starch were also significant (p < 0.05) on migration rate. There was significant (p < 0.05) interaction effect of starch and oil on migration rate.
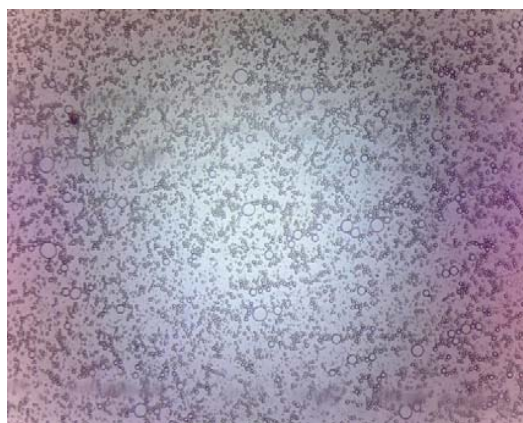
(a)
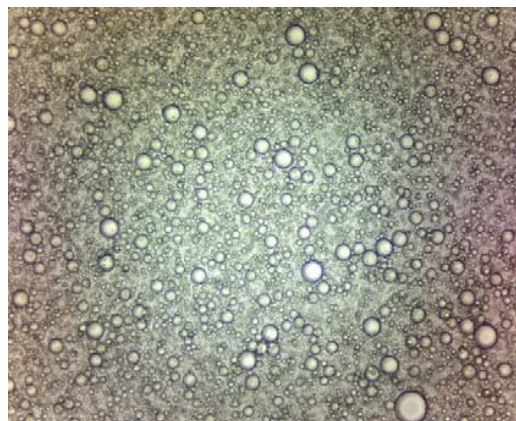
(b)
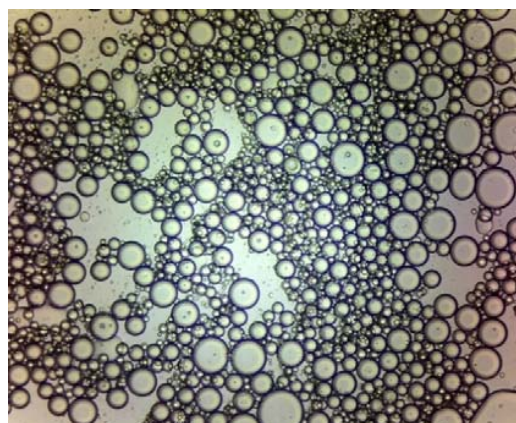
(c)

Fig. 4 Photomicrographs of BGNS-stabilized emulsions with (a) concentrated o/w emulsions showing flocculation; (b) growth in droplet diameter from (a); and (c) visible signs of phase separation

The relationship between the BGNS and oil with respect to migration rate is given by (2).

$$MR = 0.13358 - 0.068905\,X_1 + 4.97721 \times 10^{-3}X_2 - 1.34625 \times 10^{-3}\,X_1X_2 + 9.17156 \times 10^{-3}X_1^2 + 3.04640 \times 10^{-5}X_2^2 \qquad (2)$$

where MR = migration rate (mm/min), X_1 = BGNS (% w/v), X_2 = oil (% w/v), X_1X_2= interactive effects of BGNS and oil,X_1^2 = quadratic effect of BGNS, X_2^2= quadratic effect of Oil.

The detail of the impact of each emulsion variables is given in (2). Increasing BGNS decreased migration rate whereas increase in oil increases migration rate significantly. Their interactive effect was negative which means a little increase in either of the two will have greater negative effects on the migration rate. Hence, the quadratic effect is positive on the migration rate. Both encourage emulsion stability by lowering the migration rate and this can be seen from Fig. 6.
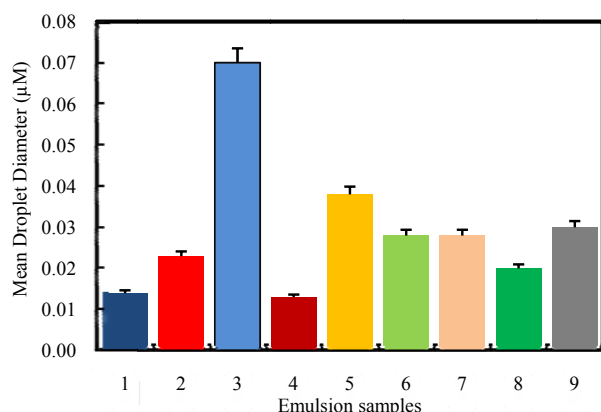
Fig. 5 The absolute mean droplet sizes of the BGNS-stabilized emulsions as observed on Day 1: 1 = 1% w/v BGNS +5% w/v oil; 2 = 1% w/v BGNS + 30% w/v oil; 3 = 1% w/v BGNS + 55%% w/v; 4 = 3% w/v BGNS + 5% w/v oil; 5 = 3% w/v BGNS + 30% w/v oil; 6 = 3% w/v BGNS + 55% w/v oil; 7 = 5% w/v BGNS + 5% w/v oil; 8 = 5% w/v BGNS + 30% w/v oil; 9 = 5% w/v BGNS + 55% w/v oil

G. Optimum BGNS for a Stable Emulsion

The numerical optimization with objectives to minimize migration rate in the range of starch and oil was carried out and the emulsion with 5% w/v BGNS and 30% w/v oil with desirability was selected as the optimal.

The MR obtained for the BGNS stabilized emulsion (0.0021 ± 0 .0003mm/min to 0.4400 ± 0.0150mm/min) was higher than the corresponding BGNF emulsion also, the mean droplet sizes of the BGNS-stabilized emulsions (0.100 ± 0.0002μm to 0.0700 ± 0.0003μm) are much higher than the corresponding BGNF-stabilized emulsions. The MR and mean droplet size values were significantly different ($p < 0.05$) in both cases.

TABLE V
ANALYSIS OF VARIANCE (ANOVA) FOR THE EFFECT OF BAMBARA GROUNDNUT STARCH AND OIL ON THE EMULSION MIGRATION RATE

| Source	Sum of squares	DF	Mean square	F-value	p-Value prob F
Model	0.25	5	0.050	604.41	0.0001
Starch	0.079	1	0.079	959.70	0.0001
Oil	0.033	1	0.033	400.76	0.0001
Starch * Oil	0.032	1	0.032	391.52	0.0001
Starch^2	4.554E-003	1	4.554E-003	55.24	0.0007
Oil^2	6.042E-004	1	6.042E-004	7.334	0. 0424
Residual	4.123E-004	5	8.245E-005		
Lack of Fit	2.058E-004	2	1.029E-004	1.49	0.3545
Pure Error	2.065E-004	3	6.883E-005		p
Cor Total	0.25	10			
R^2	0.9983				
Adj R^2	0.9967				
C.V	7.02				
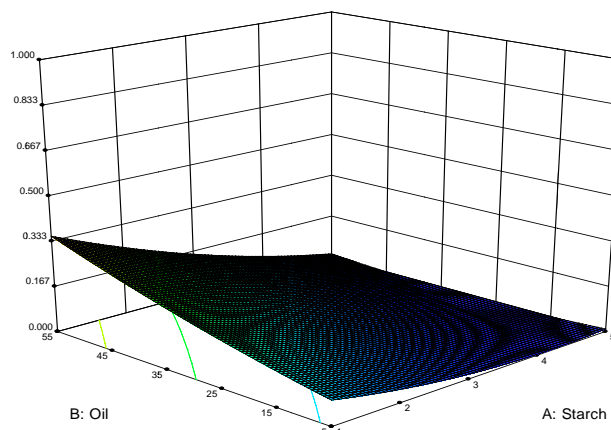
Fig. 6 The effects of BGNS (%w/v) and oil (%w/v) concentration on the droplet migration rate of the BGNS-stabilized emulsion

H. Effects of Storage Time on the MR and Droplet Size of the BGNF-Stabilized Emulsion

Storage time had no significant ($p > 0.05$) effect on the MR of BGNF-stabilized emulsions but the droplet size was significantly ($p < 0.05$) different. The storage time significantly ($p < 0.05$) increased the droplet size but not MR. This indicates that the influence of storage time on droplet size differs but the storage time has no significant ($p > 0.05$) effect on the MR.

Fig. 7 showed particle size analysis for the optimal emulsion containing 9% w/v BGNF and 39% w/v oil. The particle size growth over the days was minimal and the combination provided an optimal oil and water stabilized by BGNF.

The droplet size was least on day 1 (0.0385±0.0021μm) and highest on day 5 with droplet size 0.0075 ± 0.00070μm, while the overall mean droplet size over the storage duration period was 0.0592 ± 0.0139μm. There appeared to be a linear response to the increase in the droplet size of the BGNF-stabilized emulsion with increased storage duration.

The droplet size obtained on day 2, day 3 and day 4 were also not significantly different ($p > 0.05$). The droplet size noticed on day 3, day 4 and day 5 were also not significantly different ($p > 0.05$). However, droplet size on day 1 increase significantly ($p < 0.05$) to day 5.

The increase in the MR (0.0134 ± 0.0039mm/min) and the mean droplet size 0.0385 ± 0.0021μm on day 1 to the MR value (0.0283 ± 0.0042) and mean droplet size (0.05920 ± 0.00139μm) on day 5 is not significantly ($p > 0.05$) different. This fits into the hypothesis that the BGNF stabilized emulsions are stable with storage time. Most experimental emulsions destabilize in hours. The BGNF-stabilized emulsions were stable for 5 Days when they were discarded. This is despite the absence of any co-stabilizer. The trend of destabilization suggests that the emulsions will survive for longer days. Future work should be aimed at incorporating a preservative into the emulsion to study the stability beyond the duration used in this work.
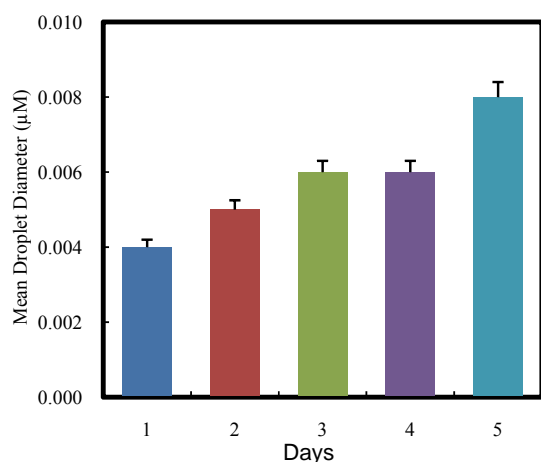
Fig. 7 The absolute mean droplet sizes of the emulsion containing 9% w/v BGNF and 39% w/v oil as affected by storage time

I. Effects of Storage Time on the MR and Droplet Size of the BGNS-Stabilized Emulsion

Storage time had significant ($p < 0.05$) effect on the MR and droplet size of BGNS-stabilized emulsions (Figs. 8 and 9). The storage time showed significant ($p < 0.05$) increase in the MR and droplet size. This indicates that the influence of storage time differs. The emulsion containing 5% w/v BGNS and 30% w/v oil was preferred because it has a very low droplet migration rate and good physically observed stability.

The MR of this BGNS-stabilized emulsion observed on day 1 was significantly different ($p < 0.05$) from the MR value of the emulsion after day 2. The MR values for storage duration between day 2 to day 5 were however not significantly different ($p > 0.05$) from one another. Thus, the storage time has no significant ($p > 0.05$) effects on the BGNS stabilized emulsion. The particle sizes were generally higher than those observed in BGNF-stabilized emulsions. For example, the mean MR of BGNS-stabilized emulsion ranged from 0.0124 ± 0.0013mm/min on day 1 to 0.0483 ± 0.0130mm/min for the storage period of day 5. The overall mean MR value over the 5 day period was 0.0370 ± 0.0147 mm/min (Fig. 8).

The mean droplet size of starch emulsion also increased over the storage duration from 0.0195 ± 0.0070μm to 0.0340 ± 0.0028μm with an overall mean of 0.027 ± 0.0055μm over the storage duration. There was cluster homogeneity in droplet size within inter-day storage period. For instance, there was no significant ($p > 0.05$) difference between the droplet size obtained for starch droplets after day 1 and day 2 storage; day 2 and day 3 storage; day 3 and day 4 storage; and day 4 and day 5 storage (Fig. 9). However, the droplet size were significantly ($p < 0.05$) different over an extended period of 48 hr i.e. between day 1 and day 3; day 2 and day 4; and day 3 and day 5.

The rate of instability on the subsequence storage days was higher than those observed in BGNF-stabilized emulsions.

The current findings revealing the ability of BGN to stabilize emulsions generally agree with earlier findings. Eltayeb and others [22] reported that BGNF demonstrated

water-absorption capacity of 281.35% while its protein isolate has 221.83% capacity; the protein isolate demonstrated 210% foaming capacity at pH 9.0, and emulsion stability of 70% after 48h. Lawal and co-workers [24] investigated the functional properties of native and chemically modified protein isolates of BGN. The results indicated an initial increase in emulsifying activity with increase in protein concentration. Both acetylation and succinylation improved the emulsifying stability of the native protein.

Although this emulsifying property was found with the protein isolate, the result of the current study suggests that the whole flour may be a better emulsifier than the isolated protein. Whole legume flours have demonstrated functional properties including emulsifying activity attributed to their dynamic protein and carbohydrate blend [25].

Native starch has limited use in food products depending on the properties exhibited. Starches derivatives, also called modified starch are thus made through physical, enzymatic or chemical treatment of native starch in order to enhance their functional properties including improvement in texture, stability to temperature and pH. Such modification makes modified starches popular as thickening agents (viscosity modifiers), emulsifiers, tablet disintegrant and binder in paper industries. Thus, modified starch is used for example as toppings for pizza where it thickens upon heating and less viscous when cooled. Modified starch is also used as emulsifier in French dressing.

While native starch is seldom used as emulsifiers, a number of patented works have shown the good emulsifying properties of modified starch [26], [27]. In the current study, the BGNF-stabilized emulsions generally demonstrated double the stability of the BGNS-stabilized ones. There are good reasons to suggest that the protein component of the BGNF contributes significantly to the emulsifying superiority of BGNF. This is because, isolated BGN protein showed 70% emulsifying capacity in 48 hours in earlier study [22]. BGNF however, has demonstrated higher emulsifying property, having stabilized emulsion for long (up to Day 5).

Both BGNF and BGNS have demonstrated to varying degrees, the potential to emulsify emulsions with prospects for use in commercial food and pharmaceutical products.
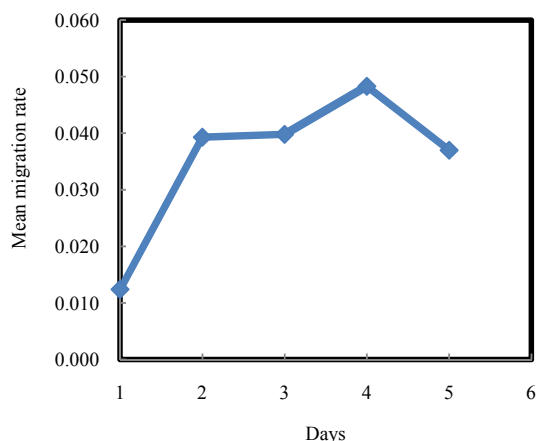
Fig. 8 The mean migration rate of the emulsion containing 5% w/v BGNS and 30% w/v oil as affected by storage time
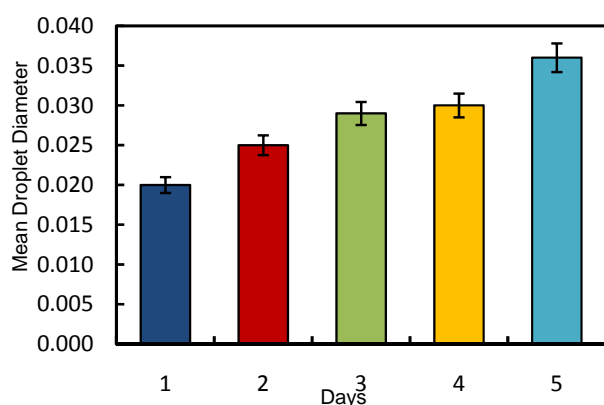

Fig. 9 The absolute mean droplet sizes of the emulsion containing 5% w/v BGNS and 30% w/v oil as affected by storage time

IV. CONCLUSION

In line with the set objectives, this study has been able to assess the ability of BGNF and BGNS to stabilize oil-in-water emulsions. An emulsion containing 9% w/v BGNF and 39% w/v oil provide optimal stable characteristics. For BGNS-stabilized emulsions, 5% w/v BGNS and 30% w/v oil gave an optimal emulsion. In both cases, the emulsions were stable till day 5.

In general, BGNF appeared to be a better emulsifier than the BGNS. This may be due to the multiple constituents of starch, protein and ash in BGNF compared to BGNS which contains starch only. The hypothesis tested in this study is valid as both BGNF and BGNS demonstrated emulsifying properties.

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